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Aliphatic polyesters with pendent unsaturation and poly(ethylene glycol) groups : synthesis, characterization, and encapsulation studies.

Bryan K. Parrish
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ALIPHATIC POLYESTERS WITH PENDENT UNSATURATION AND POLY(ETHYLENE GLYCOL) GROUPS: SYNTHESIS, CHARACTERIZATION, AND ENCAPSULATION STUDIES

A Dissertation Presented

by

Bryan K. Parrish

Submitted to the Graduate School of the
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DEDICATION

In memory of my grandfather Kenneth Lester Parrish who taught me to ski and fish, shared his name and his birthday with me, and took the time to talk chemisty with me.

ACKNOWLEDGMENTS

First and foremost I thank my parents Jim and Ione Parrish. They raised me to take pride in my work, to be dedicated, and to persevere despite the obstacles that life places in my path. Their unconditional love and support has given me the courage and strength to be successful so far from home. I thank the rest of my family especially my sister, brother-in-law, nieces, and grandparents for making home such a wonderful place to come back to. Looking forward to holidays spent with family has gotten me through some tough times and I always returned from visits home refreshed and ready to face the challenges of my research. I thank Jen Simeone for her love and friendship and for rescuing me at a point in my life when I didn't even know I needed to be saved. I am grateful for the time we spent together and will never forget the adventures of PB&E. I also thank Jen's parents Mike and Lynne for welcoming me into their family and giving me a place in Massachusetts to call home. I thank Jim Goldbach for being a true friend. I have needed someone to lean on many times over the past several years and he has never let me down. I thank Marilyn Durrett for her unwavering confidence in my abilities and for encouraging me since high school. I thank all the friends I had the pleasure of meeting in graduate school especially Kevin W., Jen C., Kevin S., Greg, Drew, Brad, Liz, and Sang-Ho. All the great times we shared outside the lab brightened my life and helped keep me sane. I would also like to thank my committee and my advisor for their scientific guidance and inspiration. Lastly, I thank the Emrick research group especially Habib, Rui, Ken, Chip, and Kevin who were there at the beginning and Rebecca who helped me make it through to the end.

ABSTRACT

ALIPHATIC POLYESTERS WITH PENDENT UNSATURATION AND POLY(ETHYLENE GLYCOL) GROUPS: SYNTHESIS, CHARACTERIZATION, AND ENCAPSULATION STUDIES

MAY 2006

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Directed by: Professor Todd S. Emrick

Aliphatic polyesters are widely used in biomaterial applications including drug delivery and tissue engineering because they are both biocompatible and biodegradable. However, these materials are primarily semi-crystalline, hydrophobic, solids at biologically relevant temperatures and lack functionality for altering their properties. Consequently methods to incorporate functional groups pendent to aliphatic polyesters and thereby tune their properties are sought. Pendent functionalization presents a significant challenge as the degradable nature of the polyester backbone severely limits the chemistry that can be performed without inducing degradation. The focus of this thesis is the preparation of pendent-functionalized aliphatic polyesters by controlled methods to give well-defined materials with a diverse range of functional moieties and physical properties.

Pendent functionalization was achieved through the synthesis of α-substituted lactone monomers, followed by Sn(II) mediated ring-opening polymerization and post-polymerization modifications. Lactone monomers substituted with allyl, cyclopentene,

and acetylene groups were prepared and their homo-/copolymerization chemistry was studied. Post-polymerization modifications that could be achieved without degradation or cross-linking were explored to give polyesters with dramatically altered properties relative to conventional aliphatic polyesters. Water-soluble PEG-grafted polyesters, as well as, polyesters with pendent oligopeptide sequences and drug moieties were prepared. The suitability of these materials for biomaterial applications was evalutated by cytotoxicity testing using mouse fibroblasts and human red blood cells. The application of the pendent-functionalized polyesters as polymer-drug conjugates and cross-linked microparticles was also explored.

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CHAPTER 1

ALIPHATIC POLYESTERS AS BIOMATERIALS: BACKGROUND, SYNTHESIS, AND FUNCTIONALIZATION STRATEGIES

1.1 Introduction

Early efforts to use synthetic polymers as biomaterials were based on high volume commodity polymers such as polyurethanes, polyacrylates, nylon, and poly(tetrafluoroethylene); polymers that were clearly designed for other purposes. Nevertheless, the wide availability and favorable physical properties of these polymers led to their use by surgeons in medical procedures that required durable and inert materials.[1-2] While many advances were achieved using such polymers in surgical and other biological applications, these materials were also found to elicit significant problems, such as inflammation and infection, due to the immune response of the body to the presence of foreign materials. Notable pioneering examples include the efforts of DeBakey and coworkers, who as early as the 1950's used Dacron™ (polyethylene terepthalate) for cardiovascular prostheses.[3] These procedures were found to be successful in the repair of large arteries, but failed in cases where the internal diameter was less than 5 mm. The breadth of expertise needed to solve such challenging problems in biomaterials, and effectively introduce synthetic materials to the body, includes chemistry, biology, engineering, and clinical surgery, thus generating a challenging interdisciplinary field that requires collaborative activity among these disciplines.

While many different types of polymers are of interest for biomaterial applications, aliphatic polyesters are particularly relevant due to their degradation behavior under physiologic conditions, which makes them desirable for resorbable applications. Aliphatic polyesters were initially used to fabricate degradable sutures in the 1960's [2] and have since found use in a wide range of biomaterial applications including drug-delivery systems,[4] tissue-engineering scaffolds,[5] and temporary tissue/bone replacement[6] as depicted in Figure 1.1.

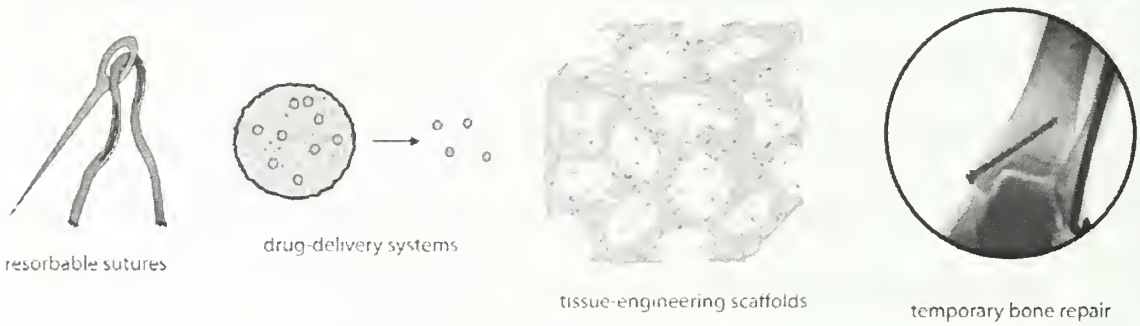

Figure 1.1 Examples of biomaterial applications using aliphatic polyesters

Recent advances in the field include the work of Langer on aliphatic polyesters for tissue-engineering and drug-delivery,[7] Fréchet and Grinstaff on drug-delivery[8] and surgical[9] applications of dendrimers, and Duncan in the area of drug-delivery systems and polymer therapeutics.[10]

1.2 Synthesis of aliphatic polyesters

For medical applications polymers with known end-groups, controlled molecular weight, and narrow polydispersity (PDI, defined as M_w/M_n) are desired such that their *in vivo* properties and degradation behavior can be accurately predicted and reproduced. An effective approach to the controlled synthesis of aliphatic polyesters

entails the ring-opening polymerization of cyclic (di)esters including lactide, glycolide, ε-caprolactone (ε-CL), and δ-valerolactone (δ-VL) (Figure 1.2). Polyesters can also be prepared by condensation or bacterial polymerization.[11-12] Most notable is the commercial production of poly(hydroxyalkanoates) by Metabolix, Inc.[13-14] Through metabolic engineering of bacteria, polyesters with a range of mechanical and physical properties can be prepared that are suitable for packaging applications and medical devices.[15] However these materials have broad PDIs and poorly defined end-groups and are thus beyond the present focus on controlled synthetic methods. The ring-opening homo- and copolymerization of lactones and lactides is performed in the bulk or in solution using organometallic catalysts such as aluminum iso-propoxide ($Al(OiPr)_3$), tin(II) 2-ethylhexanoate, ($Sn(Oct)_2$), tin(II) trifluoromethane sulfonate ($Sn(OTf)_2$), as well as organic catalysts based on N-heterocyclic carbenes.[16-17] Primary and secondary alcohols or amines are used to initiate polymerization which proceeds in a well-controlled fashion by means of a coordination-insertion mechanism.

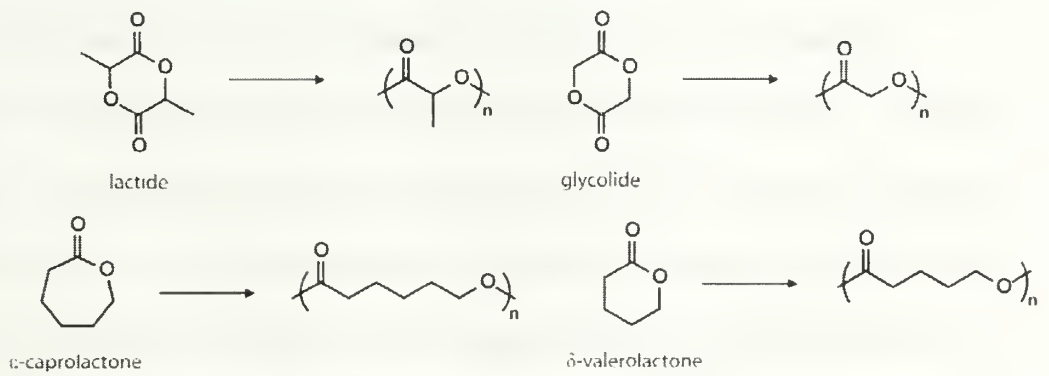

Figure 1.2 Aliphatic polyesters prepared by ring-opening polymerization of lactides and lactones

Aliphatic polyesters exhibit very different physical and mechanical properties depending on the structure of the repeat unit. For example, poly(glycolide) is highly crystalline and exhibits a melting temperature (T_m) of 220-225 °C and a glass transition temperature (T_g) of 36-40 °C and has very limited solubility. At room temperature poly(glycolide) is above T_g and thus a very stiff material with a modulus of 7.0 GPa and elongation of only 15-20% making it best suited for applications such as temporary bone repair which require high strength, load bearing materials. Poly(ε-CL), in contrast, is much more flexible with $T_m \sim 60$ °C, $T_g \sim -60$ °C and a modulus and elongation at room temperature of 0.4 GPa and 300-400% respectively. Such materials are best suited for non-load bearing applications that require more rapid degradation such as drug-delivery and tissue-engineering. By copolymerization, a range of mechanical properties and degradation rates can be achieved, as is observed for copolymers of glycolide and ε-CL which exhibit the high strength of poly(glycolide) as well as the increased solubility and flexibility of poly(ε-CL) making them ideal for monofilament sutures.[2]

Despite the range of mechanical properties and physical properties that can be achieved, all of these aliphatic polyesters are semi-crystalline, hydrophobic solids at physiologic temperatures, and are lacking in functionality for altering these properties. Thus, methods to integrate functionality into aliphatic polyesters to tailor their physical and biological properties have been sought. Water soluble polyesters are of particular interest for injectable applications, as are polyesters functionalized with drug moieties, cell-adhesion promoters, and targeting oligopeptide sequences for drug-delivery and tissue-engineering applications.[18-22]

1.3 Strategies for polyester functionalization

Functionalization of aliphatic polyesters is a delicate challenge from a synthetic perspective, as their degradable nature that makes them desirable as biomaterials also limits the types of chemistry that can be used for their modification. Consequently, mild synthetic strategies must be employed for controlled functionalization that can proceed in the absence of ester bond degradation. Aliphatic polyester functionalization can be viewed from a macromolecular architecture perspective, including end-group functionalization of linear polyesters, non-linear polyesters such as dendritic and hyper-branched polymers that contain multiple functional groups as chain-ends, and the introduction of pendent functionality distributed as grafts on linear polyester backbones to give functional comb-type structures.

1.3.1 End-group functionalization

The simplest means of functionalizing aliphatic polyesters is at the chain-ends (Figure 1.3). This can be achieved using functional initiators for ring-opening polymerization, or through end-capping reactions. Numerous reports describe the initiation of lactone polymerization from the chain-end hydroxyl groups of poly(ethylene glycol) (PEG) -diols and -monomethyl ethers, to produce hydrophilic and often water soluble tri- and di-block copolymers, that can assemble into micellar structures in water with a polyester core and a PEG corona.[23-24] Examples of polyester functionalization by end-capping include esterification of the polyester hydroxyl chain-end with 4-azidobenzoyl chloride to give UV-photo-curable polyesters,[25] and end-

capping with phosphorylcholine residues to give polyesters with phospholipid like moieties that reduce protein adsorption relative to non-functionalized polyesters.[26]

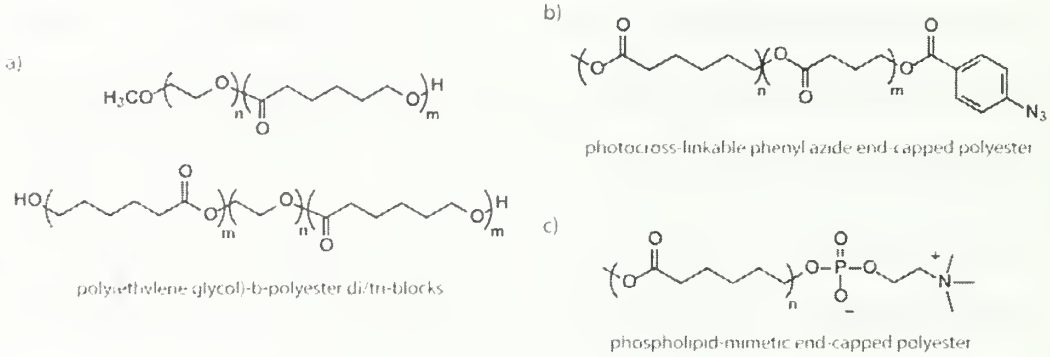

Figure 1.3 End-functionalized polyesters a) poly(ethylene glycol)-b-polyester, b) phenyl azide end-capped, and c) phosphorylcholine end-capped

Chain-end functionalization has advantages for its simplicity, but clearly limits functional group incorporation to low levels (one or two functional groups per polyester molecule). Moreover, during polyester degradation by cleavage of ester bonds in the backbone, very little of the degraded material remains bound to the functional group of interest. Thus, methods to increase the level of functionality on polyesters have been pursued by the synthesis of dendritic, hyper-branched and comb-like polyester-based structures.

1.3.2 Highly functionalized dendritic and hyper-branched aliphatic polyesters

Non-linear polymer architectures including dendritic, hyper-branched, and linear-dendritic hybrid materials have been prepared as a means of altering polyester properties and introducing very high levels of functionality (Figure 1.4). In addition to the effect of branching on solid-state and solution properties, the large number of end-

groups that result from this branching offer the opportunity to obtain very high levels of functional group loading. Dendrimers are especially attractive for their exceptionally high level of chain-end functionality, and thus are of interest in polymer-based drug delivery, where high drug loading per delivery vehicle can be particularly beneficial. Fréchet, Szoka, and coworkers recently put this concept into practice through the synthesis of aliphatic polyester dendrimers with covalently attached drugs such as the anti-cancer drug doxorubicin (Figure 1.4a).[8,27] In vivo evaluation of such conjugates demonstrated the effectiveness of the dendritic drug carrier relative to the direct use of free small molecule drugs.

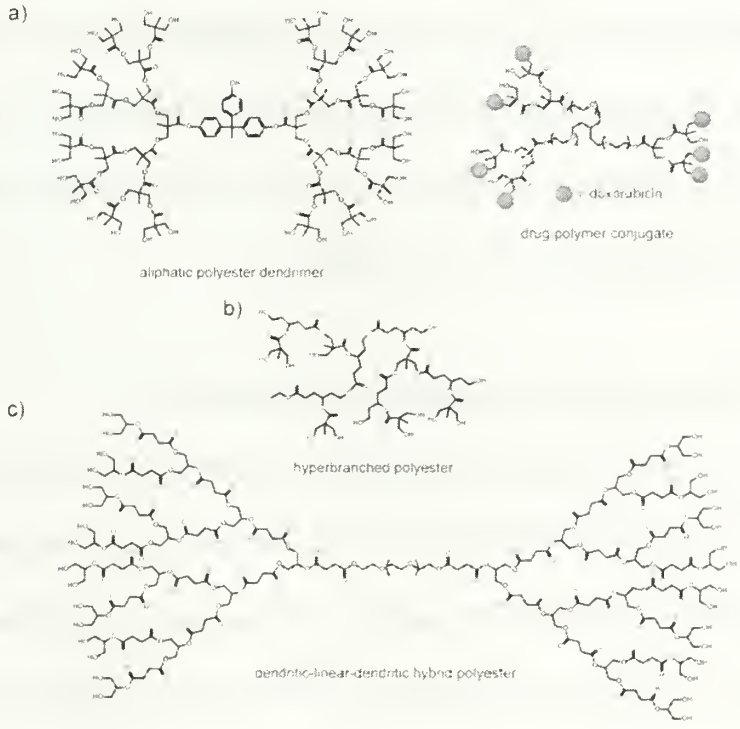

Figure 1.4 a) Polyester dendrimer and drug conjugate; b) hyper-branched aliphatic polyester; and c) dendritic-linear-dendritic triblock copolymer

Hyper-branched polymers also provide considerable branching and chain-end functionality, but can be prepared by conventional polymerization chemistry rather than

the somewhat tedious step-wise coupling approach necessary for dendrimer synthesis. Hyper-branched aliphatic polyesters have been prepared by ring-opening polymerization of lactones bearing hydroxyl groups, as seen in reports of Hedrick and coworkers on bis(hydroxymethyl)-substituted ε-CL[28] (Figure 1.4b) and Fréchet and coworkers on ring-opening polymerization of hydroxyethyl-substituted ε-CL.[29] Hyper-branched poly(ε-CL) samples of high molecular weight (65,000-85,000) were found to be soluble in polar solvents such as DMSO and methanol, due to the large number of hydroxyl end-groups in the structures. Hybrid copolymers consisting of linear and dendritic segments have also been prepared and evaluated as biomaterials, for example by Grinstaff and coworkers on photo-cross-linkable dendritic-linear-dendritic triblocks (Figure 1.4c) prepared for ophthalmic tissue repair applications.[9] After methacrylate end-capping, the hybrid copolymers were cross-linked to seal corneal lacerations and found to perform better than nylon sutures.

1.3.3 Aliphatic polyesters with pendent functionality

Functionalization of linear aliphatic polyesters with grafted moieties placed pendent to the polymer backbone has also been explored to integrate the desired functionality into the polymer material, while maintaining the linear backbone. As the introduction of pendent functionality to commercially available aliphatic polyesters presents few synthetic options and carries the risk of polymer degradation, recent work has focused on ring-opening polymerization of functionalized lactones and lactides. While simple functional groups that are compatible with lactone ring-opening polymerization conditions can be introduced with relative ease, many substituents

present difficulties, indicated for example by Jérôme and coworkers where PEG-1,000 g/mol substituted ε-CL gave low PEG-grafting densities by ring-opening polymerization.[30] Limited or non-polymerizability of functionalized lactones can be due to steric hindrance imposed by the functionality, incompatibility of the functional group with the catalyst, or simply lactone stabilization resulting in insufficient ring strain for polymerization.

The polymerization of any monomer requires a negative free energy change $\Delta G°$, the enthalpic and entropic factors of which are described by the Gibbs' free energy equation $\Delta G° = \Delta H° - T\Delta S°$. Ring-opening polymerization carries a negative entropy term due to lower translational entropy of the polymer backbone relative to the monomer. Thus, for $\Delta G°$ to be negative, the enthalpy change for polymerization $\Delta H°$, which is negative, must be large enough to outweigh the entropic term. For cyclic monomers, $\Delta H°$ is related to ring strain, which is a combination of the steric crowding, bond angles, and bond lengths imposed by the ring conformation. Generally, 5-membered lactones exhibit negligible ring strain, giving a small $\Delta H°$ and a positive $\Delta G°$ such that homopolymerization to appreciable molecular weight is not observed. The higher ring strain that is present in 6- and 7-membered lactones affords a larger $\Delta H°$ and corresponding negative $\Delta G°$ allowing for homopolymerization under a variety of conditions.[31]

The decrease in the number of gauche interactions in a polymer backbone relative to the ring conformation of the monomer also contributes to the magnitude of $\Delta H°$. The greater the decrease in gauche conformations between monomer and polymer conformations, the greater the $\Delta H°$ associated with the conformation change.[32]

Substitution generally decreases the polymerizability of lactones because it increases the number of gauche interactions in the polymer, lowering the relative decrease in gauche interactions between monomer and polymer conformations, and thus lowering ΔH°. For 7-membered lactones this effect does not outweigh the contribution due to ring strain, however in 6-membered lactones, where ΔH° due to ring stain is smaller, substitution can render them non-polymerizable.

Polymerization is also limited by the ceiling temperature (T_c) and the equilibrium monomer concentration ($[M]_e$). The ceiling temperature is defined as the temperature above which depolymerization is favored over propagation. The equilibrium monomer concentration is the relative concentration of monomer that exists at equilibrium. The relationship between ceiling temperature and equilibrium monomer concentration is given in Equation 1.1 where R is the gas constant and T_c is given in Kelvin.

$$T_c = \frac{\Delta H^{\circ}}{\Delta S^{\circ} + R \ln [M]_e}$$

Equation 1.1

In order to achieve an appreciable degree of polymerization, at a given temperature the monomer concentration must be greater than $[M]_e$, or conversely at a given monomer concentration the temperature must be lower than the ceiling temperature for polymerization to occur.

Since the incorporation of complex functional moieties at the monomer stage can limit polymerizability due to steric hindrance, incompatibility, and reduced ring

strain, a stepwise approach is preferred (Figure 1.5). In such an approach a simple and compatible functional group is incorporated at the monomer stage, followed by ring-opening polymerization, and finally post-polymerization modification to yield polyesters with more diverse and complex pendent functional groups.

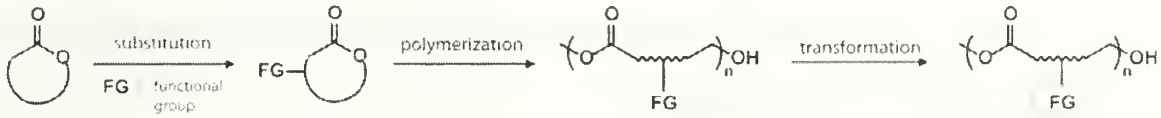

Figure 1.5 Stepwise approach to polyester functionalization

By copolymerization of functionalized lactones with conventional lactone monomers, the functional group density can be tailored over a wide range. The ability to produce substituted polyesters for subsequent modification opens many options for further functionalization that are not possible in the case of unsubstituted polyesters. However, the conditions for post-polymerization reactions must be chosen carefully to avoid degradation or cross-linking. Many functional polyester structures have been reported, including those with pendent alkyl bromides,[32-33] ketones,[34] alcohols,[35] alkenes,[33] carboxylic acids,[35] acrylates,[36] 2-bromo-2-methylpropionates,[37] PEG chains,[30+38] and dendrons[39].

1.4 Recent studies on pendent functionalization of aliphatic polyesters

The synthetic versatility associated with lactone chemistry offers a number of methods for introducing functionality into the lactone ring. The most commonly employed methods for producing functionalized lactones that are amenable to ring-opening polymerization include: 1) Baeyer-Villiger ring-expansion of α-substituted

cyclohexanones; 2) mono-substitution of 1,6-cyclohexane diol followed by oxidation with pyridinium chlorochromate (PCC), and subsequent ring-expansion using Baeyer-Villiger chemistry; and 3) substitution α to the carbonyl of the lactone, using lithium diisopropylamide (LDA) as a non-nucleophilic base, followed by addition of an appropriate electrophile. Taken together, these routes can be used to produce variously substituted ε-CL and δ-VL monomers, each having its own attractive features in terms of synthetic ease and versatility.

Hedrick, Jérôme, and coworkers provided early examples of pendent functionalized poly(ε-CL) by polymerization of allyl-functionalized ε-CL (**1**).[33] This new lactone monomer was prepared from 2-allyl cyclohexanone by Baeyer-Villiger oxidation with meta-chloroperoxybenzoic acid (m-CPBA) (Figure 1.6). This oxidative ring-expansion gives lactone **1**, with the expected oxygen insertion between the carbonyl group and adjacent methine. Olefin epoxidation by m-CPBA complicates the synthetic method to some degree resulting in an overoxidized side-product. Nonetheless, lactone **1** can be separated from the side-product and used effectively in ring-opening polymerization.

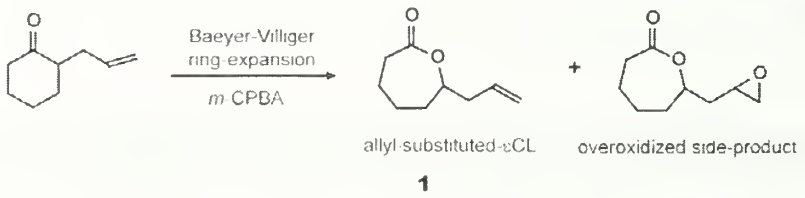

Figure 1.6 Synthesis of allyl-functionalized ε- CL (1) by Baeyer-Villiger oxidation of 2-allylcyclohexanone

Using a variety of hydroxyl containing initiators, lactone **1** was homopolymerized and copolymerized with both ε-CL and L,L-lactide using $Sn(Oct)_2$ as

the catalyst to give allyl functionalized polyesters shown as (**2**) (Figure 1.7). In all cases, the experimental and theoretical molecular weights were in good agreement over the range of 3,000-12,000g/mol, and the polydispersities were fairly narrow (1.1-1.4), in accord with a controlled polymerization, and in clear contrast to typical polydispersity values of 2 for polyesters prepared by polycondensation. Homopolymer **2** was found to be an amorphous material with a glass-transition temperature (Tg) of -62 °C, compared to -60 °C for poly(ε-CL).

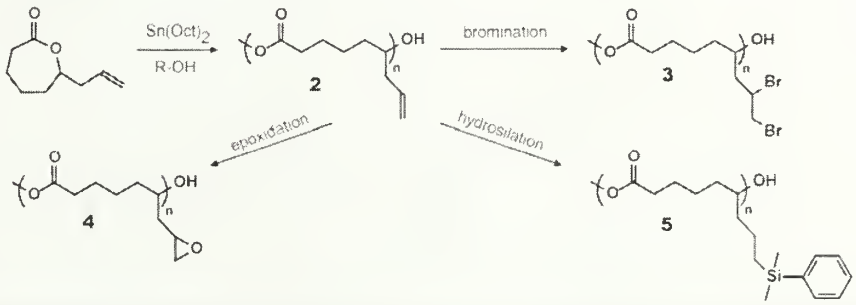

Figure 1.7 Polymerization of lactone 1 and subsequent functional group transformations: bromination (3), epoxidation (4), and hydrosilation (5)

Chemical transformations performed on the pendent allyl groups of polymer **2** included bromination, epoxidation, and hydrosilation reactions to give polyesters (**3-5**) respectively. In all cases the transformations were achieved in the absence of degradation or cross-linking, as shown by comparing gel permeation chromatography (GPC) traces of the starting polyesters and the products. This work nicely demonstrates the compatibility of unsaturated functional groups incorporated into lactone monomers with controlled ring-opening polymerization, as well as a multi-step approach to polyester functionalization that can bring synthetic diversity to aliphatic polyesters upon post-polymerization modification.

Hedrick and coworkers further expanded the diversity of pendent functionalized polyesters by the synthesis of hydroxyl and carboxyl derivatives.[35] Incorporation of these moieties into the polyester structure required the synthesis and polymerization of protected ε-CL derivatives shown in Figure 1.8.

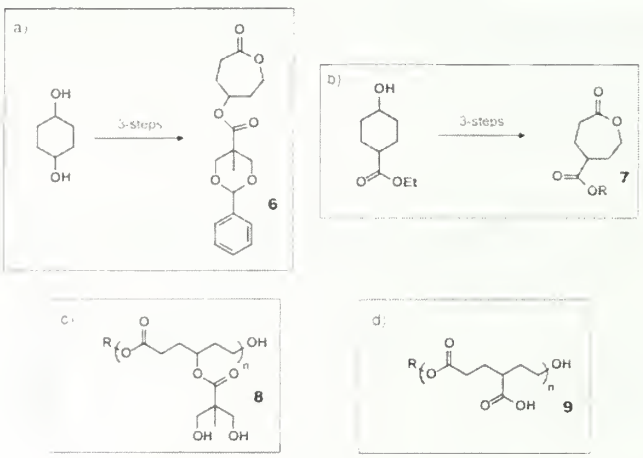

Figure 1.8. Protected hydroxyl and carboxyl monomers and deprotected polymers

Protected hydroxyl derivative (**6**) was prepared from 1,6-cyclohexane diol, by mono-substitution with 2,2'-bis(phenyldioxymethyl) propionyl chloride, followed by oxidation with PCC, and Baeyer-Villiger ring expansion. Protected acid derivative (**7**) was synthesized from ethyl-4-hydroxycyclohexane carboxylate by oxidation with PCC, cleavage of the ethyl group with H_2SO_4, protection with benzyl bromide, and ring expansion with m-CPBA.

Homo- and co-polymerizations of monomers **6** and **7** were performed using $Sn(Oct)_2$ catalysis, and ε-CL as the comonomer, to afford the corresponding aliphatic polyesters bearing protected pendent functional groups. The benzylidene protecting groups were removed by catalytic hydrogenolysis to afford the desired hydroxyl (**8**) and

carboxyl (**9**) pendent functional groups. Importantly, this deprotection strategy proved compatible with the aliphatic polyester backbone, such that the functionalized polymers could be prepared cleanly.

While these examples nicely demonstrate a stepwise approach to controlled functionalization of aliphatic polyesters the chemistry involved in each case is often tedious and specific to a single type of functional group. In addition, the polymerization chemistry of lactones with substituents α to the carbonyl was previously unexplored in the literature. Consequently, the goal of this thesis work is to develop a general method, using α-substituted lactones, whereby a diverse range of functional moieties can be introduced pendent to aliphatic polyesters through a single type of chemistry. Aliphatic polyesters with pendent PEG chains, oligopeptide sequences, and drug moieties are targeted.

1.5 Drug-delivery applications

Due to the highly toxic nature of anti-cancer drugs and the extensive negative side effects associated with conventional chemotherapy, methods for controlled delivery have received a great deal of attention over the past several decades.[40] In addition to providing more constant, therapeutic levels of anti-cancer drugs while decreasing their systemic toxicity, controlled delivery systems have been used to improve the solubility of hydrophobic drugs, enhance plasma residence times by decreasing phagocytic and renal clearance, and stabilize anti-cancer drugs in plasma so that greater concentrations of active drug can be delivered to tumor sites at lower dosing levels. Controlled drug-delivery systems can also provide sustained release of a drug

over long periods of time thus eliminating the need for frequent administration. As drug delivery systems have evolved and the synthetic methods for preparing them have been improved, more complex levels of control have become possible and much of the current focus is on the specific targeting of cancerous tissues and enhanced cellular uptake of anti-cancer drugs within these tissues.[41] Polymer-based drug delivery systems have been investigated to overcome many of the challenges associated with controlled drug delivery. These include polymer-drug conjugates[10+27] for endocytotic drug delivery and polymer microparticles[40] for subcutaneous, intramuscular, or oral administration.

Polymer-drug conjugates differ from other drug delivery systems in that the drug to be delivered is covalently bound to the polymer carrier. While methods for actively targeting cancerous tissue are being explored, the general strategy for endocytotic delivery relies on the enhanced permeability and retention effect (EPR). The EPR effect refers to the leaky vasculature and poor lymphatic drainage of tumors that result in the accumulation of large circulating polymers and polymer assemblies in tumor tissue.[41] The vasculature pore size in tumor tissue ranges from 100 to 780 nm, while the pore size in normal tissue is typically less than 6 nm. Consequently, polymer-drug conjugates >6nm cannot permeate normal tissue, and accumulate predominately in tumors where they become trapped due to the lack of effective lymphatic drainage in these tissues.[42] The conjugates then cross the cell membrane by endocytosis and are delivered to the lysosome where the pH drops to ~4-5. Many delivery systems thus incorporate ester and carbonate, acid-degradable linkages such that the polymer vehicle promotes circulation of the stabilized drug in the blood-stream, and then rapidly

degrades and releases the drug upon entering the lysosome. One example of such a polymer-drug conjugate delivery system was recently reported by Duncan and coworkers in their work on PEG-poly(ester-carbonate) block copolymer vehicles for the delivery of covalently bound Doxorubicin.[43] The polymer vehicle consists of a block copolymer prepared by condensation of PEG and bis(4-hydroxy)butyl maleate, to which Doxorubicin is bound via a thioether terminated PEG-Peptide spacer as depicted in Figure 1.9.

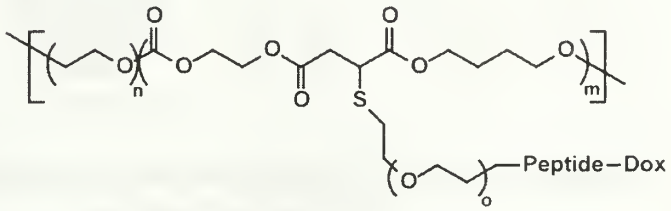

Figure 1.9 PEG-poly(ester-carbonate) block copolymer-PEG-Peptide-Doxorubicin polymer-drug conjugate

The *in-vitro* cytotoxicity and *in-vivo* antitumor activity of these conjugates was evaluated and although they released the hydrophobic drug more slowly than simple PEGylated-Doxorubicin conjugates this system may prove valuable for delivery of less hydrophobic drugs.

Polymer microparticles are generally much larger than polymer-drug conjugates (1-100μm) and are thus better suited for oral delivery to target the gastrointestinal tract, or subcutaneous/intramuscular injection for drug delivery to tissues.[44] Hydrophobic, degradable microparticles are typically prepared by emulsion/solvent evaporation techniques and used to entrap hydrophobic drugs. Once the microparticles are injected, the drug is slowly released as the particles degrade. This can provide a constant drug

release over long periods of time, and eliminate the need for frequent administration.[44] However, such drug delivery systems often suffer from a rapid initial "burst" release of drug, followed by a slow constant release, and many efforts are focused on eliminating burst release. Shenoy and coworkers have reported the use of polyester based microparticles for subcutaneous insulin delivery.[45] Insulin was physically entrapped in poly(lactide-co-glycolide) (PLGA) microspheres and then released at a constant rate upon degradation of the microspheres after injection, as depicted in Figure 1.10.

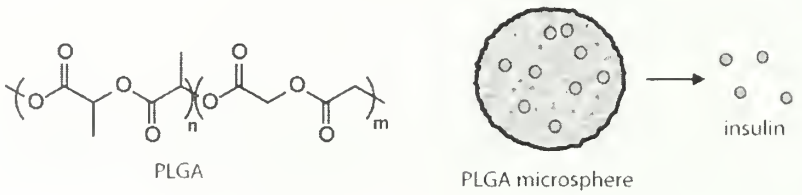

Figure 1.10 Release of insulin from poly(lactide-co-glycolide) microspheres upon subcutaneous injection

The microsphere delivery system was found to release insulin and control blood sugar levels more efficiently than plain insulin injection.

While many polymer-based systems have been investigated for polymer-drug conjugate and microparticle delivery systems, the use of aliphatic polyesters as vehicles for drug delivery and the use of functionalized aliphatic polyesters for microparticle cross-linking has been relatively unexplored. Consequently, Chapter 4 of this thesis work is devoted to polyester based polymer-drug conjugates, and cross-linked polyester microparticle applications of pendent functionalized aliphatic polyesters.

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CHAPTER 2

POLYESTERS WITH PENDENT ALKENES

2.1 Pendent allyl functionality

The approach to functionalized aliphatic polyesters presented in this thesis centers on incorporation of functionality at the lactone monomer stage. The stability of alkenes under conditions used for lactone polymerization led to the studies described in this chapter. While alkene-functionalized aliphatic polyesters are of interest for some applications, such as olefin cross-linking of methacrylates incorporated at the end-groups,[1-2] their utility in this work lies in post-polymerization functional group transformations. As described in Chapter 1, prior studies by Hedrick and coworkers on olefin-functionalized aliphatic polyesters confirmed the compatibility of alkenes with metal-mediated controlled ring-opening polymerization.[3] This chapter describes the introduction of allyl functionality α to the carbonyl group of lactones, especially δ-VL, the controlled ring-opening polymerization of the allyl-functionalized lactones, and subsequent post-polymerization chemistry of the pendent olefins.

2.1.1 Synthesis of α-allyl functionalized lactones

A general method for alkylation of lactones α to the carbonyl group was reported by Herrmann and Schlessinger in 1973,[4] and more recently described in greater detail by Mollander and coworkers in their investigation of tandem intramolecular nucleophilic acyl substitution/intramolecular Barbier cyclizations.[5] The methylene group α to the carbonyl is susceptible to deprotonation by LDA to form the

corresponding enolate, due to the acidity of the protons in this position (pKa ~ 24).[6] LDA is particularly useful for this chemistry as highly nucleophilic bases (such as *sec*-butyllithium) competitively attack the carbonyl group and result in ring-opening of the lactone. Addition of allyl bromide to the enolates in a tetrahydrofuran (THF) solution with hexamethylphosphoramide (HMPA) provides the desired allyl substitution. HMPA solvates the lactone anion and promotes reaction with allyl bromide. Careful attention to temperature, combined with slow addition of the lactone to the dilute LDA solution in THF (0.1 mM), proved critical for maximizing yield. The reaction mixture was kept at -78 °C (dry ice/acetone bath) for the lithiation and addition of allyl bromide/HMPA, then allowed to warm to -40 °C (dry ice/acetonitrile bath) to promote substitution.

The isolated yields of allyl functionalized lactones were found to increase with stability of the lactone ring. The relatively stable 5- and 6-membered lactones γ-butyrolactone (γ-BL) and δ-VL gave allyl-BL and allyl-VL in 95 and 71% yield respectively as depicted in Figure 2.1, while allyl-substitution of the less stable 7-membered ring ε-CL (due to hydrogen atom crowding)[7] was typically achieved in 40-50% yield. With respect to polymerization, allyl-VL and allyl-CL were found to polymerize readily under Sn(II) catalyzed conditions, while allyl-BL did not homo/co-polymerize.[8] Thus, initial efforts were focused on the chemistry of α-allyl-δ-valerolactone (**1**), as this monomer can be prepared in good yields, and polymerized under standard Sn(II)-mediated conditions.

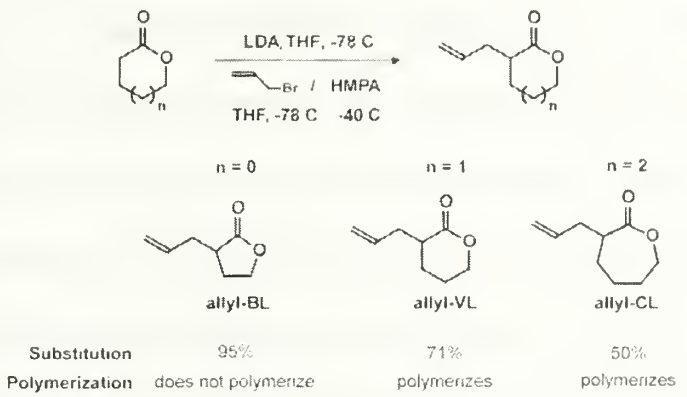


Figure 2.1 Allyl-substituted γ-butyrolactone, δ-valerolactone, and ε-caprolactone: synthesis, yield, and polymerizability

The detailed synthesis of lactone **1** is provided in Chapter 5 (experimental section), as several key experimental elements are not reported in the Mollander paper. A small amount of disubstituted lactone was always observed to form in this reaction, thus column chromatography on silica gel (0-15% ethyl acetate in hexane) was used to isolate the product. After slowly increasing the concentration of ethyl acetate to 15%, the disubstituted lactone elutes first allowing for isolation of lactone **1**. Further purification by Kugelrohr distillation gave allyl-substituted lactone **1** as a colorless, viscous liquid. Successful allyl-substitution is confirmed by the presence of olefin signals at 5.75 (CH=CH_2) and 5.06 (CH=CH_2) ppm in the ^{1}H NMR spectrum, and 135.2 (CH=CH_2) and 117.6 (CH=CH_2) ppm in the ^{13}C NMR spectrum. The intact lactone ring is confirmed by the signal at 4.26 ppm in the ^{1}H NMR spectrum, which corresponds to the δ-methylene protons (the corresponding signal for the ring-opened product is shifted upfield to 4.02 ppm), and a single peak in the carbonyl region of the ^{13}C NMR spectrum at 174.0 ppm. These results are in accord with literature values.[5]

2.1.2 Polymerization of allyl-functionalized lactone 1

Prior reports of lactones with α-allyl substitution did not address their polymerization chemistry, leaving this type of pendent functionalization of aliphatic polyesters unexplored. Allyl-substituted lactone **1** was found to undergo homo/co-polymerization both in solution (THF or toluene) and in the bulk with either $Sn(Oct)_2$ at 110 °C or $Sn(OTf)_2$ at room temperature as the catalyst. A variety of primary alcohol initiators were used successfully, including benzyl alcohol, hexanol, and ethanol (Figure 2.2a). Polymerizations were most commonly performed in the bulk at room temperature with $Sn(OTf)_2$ and EtOH to yield allyl-functionalized polyesters of type **2**.

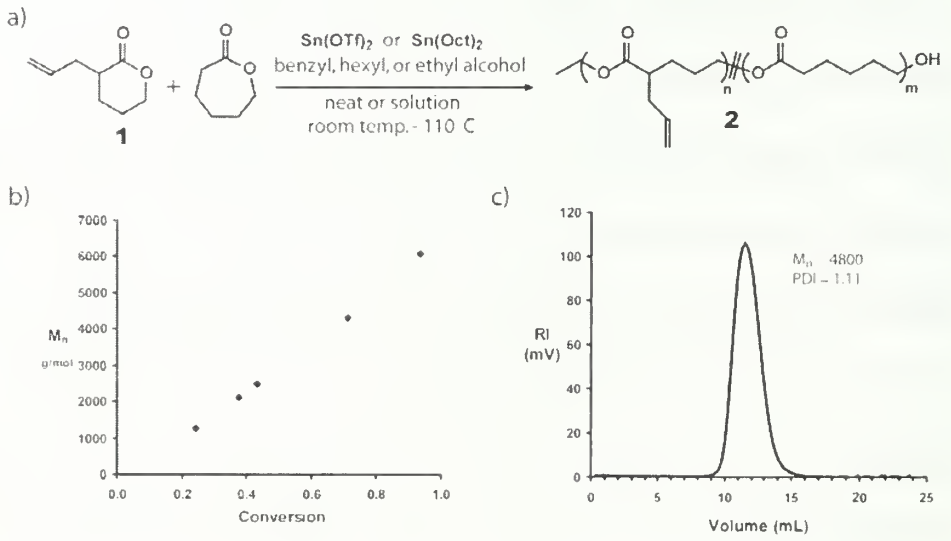


Figure 2.2 a) Schematic depiction of copolymerization of lactone 1 and ε-CL to give allyl-substituted polyesters 2; b) plot of molecular weight *vs.* conversion for homopolymerization of 1; c) GPC trace of isolated homopolymer 2 in THF

The increase in M_n with monomer conversion for the bulk homopolymerization of **1** was monitored by GPC and ^1H NMR spectroscopic analysis of aliquots taken from the polymerization mixture as a function of time. The linear increase in molecular weight

with conversion, shown in Figure 2.2b, demonstrated that introduction of the allyl group does not disrupt the controlled nature of Sn(II) mediated ring-opening polymerization. Monomer conversion of 90% or greater was typically achieved to give polyesters of type **2** with narrow polydispersities (PDI = 1.1-1.3) as determined by GPC analysis relative to polystyrene standards (Figure 2.2c). When polymerizations were allowed to proceed to full conversion, a broadening of the molecular weight distribution was observed due to competitive transesterification chemistry at high monomer conversion, which is well documented for Sn(II) mediated lactone polymerization.[9] The success of these polymerizations, and the rate at which they proceed, was found to depend critically on monomer purity and the rigorous exclusion of water. Generally, Kugelrohr distillation of the monomers was performed immediately prior to their use to remove residual water, as well as any oligomeric impurities that may have formed during storage. Glassware was flame-dried under $N_{2(g)}$, initiators were distilled over CaH_2, and catalysts were stored under dry, inert conditions. Attention to these factors generally led to high conversions and low polydispersities of the aliphatic polyesters prepared during the course of this research.

Ring-opening homo-/copolymerizations of **1** were typically run for 24 hours at room temperature, with catalyst loadings of 1-5 mole percent based on the initiator. Monomer conversion was monitored by ^{1}H NMR spectroscopy. Upon achieving greater than 90% monomer conversion, the reaction mixture was diluted in acetone and precipitated into cold methanol or hexanes to remove catalyst, and in the case of copolymerizations to remove residual unsubstituted lactone. Column chromatography was required to remove residual functionalized lactone **1**. The incorporation of **1** into

copolymers was controlled by the feed ratio of the polymerization mixture. The degree of incorporation of **1** into the polyester products, shown in Table 2.1, was calculated by integration of ^{1}H NMR spectroscopic signals, using the ratio of the allyl CH_2 signal at 5.1 ppm *vs.* the backbone -COOCH_2- signal at 4.1 ppm.

Table 2.1 Characterization of polyesters with pendent allyl functionality

Polymer	Feed Ratio (1 : ε-CL)	Incorporation (1)[a]	M_n x 10^3 g/mol[b]	PDI[b]
2_a	25 : 75	21	5.8	1.08
2_b	50 : 50	42	6.7	1.10
2_c	75 : 25	74	6.4	1.15
2_d	100 : 0	100	7.0	1.11

[a] determined from ^{1}H NMR spectra, [b] determined by GPC relative to polystyrene standards

As seen in Table 2.1, the calculated degrees of incorporation of lactone **1** into polyesters of type **2** correspond closely to the feed ratios, and in all cases polyesters with narrow molecular weight distributions were isolated. Thus the density of pendent allyl functionality in polyesters of type **2** can be tuned with a high level of precision. Typically a degree of polymerization of 60 repeat units was targeted, corresponding to molecular weights of 6-9 x 10^3 g/mol depending on the feed ratio. Maximum molecular weights of 12-15 x 10^3 g/mol were achieved, in accord with literature values for Sn(II) mediated lactone polymerization.[10-11] Attempts to achieve higher degrees of polymerization resulted in broadening of the polydispersity, and no increase in molecular weight above 15 x 10^3 g/mol, due to competing transesterification. Similar results were achieved for copolymerizations of **1** with δ-VL.

The ^{13}C NMR chemical shifts of the carbonyl groups in aliphatic polyesters are known for their sensitivity to polymer microstructure and can thus be used to determine the random or blocky structure of polyester copolymers.[12-13] The ^{13}C NMR spectra of allyl-functionalized polyester $\mathbf{2_d}$ and poly(ε-CL) homopolymers show clean carbonyl resonances at 175.2 and 173.9 ppm respectively, as shown in Figure 2.3. The carbonyl region of copolymer $\mathbf{2_c}$ (74% **1** and 26% ε-CL) shows several non-baseline separated resonances in the carbonyl region indicating a random distribution of lactones in the polymer backbone. A diblock structure or even a blocky distribution would be expected to exhibit only two baseline resolved signals, corresponding to the two homopolymer-like segments.

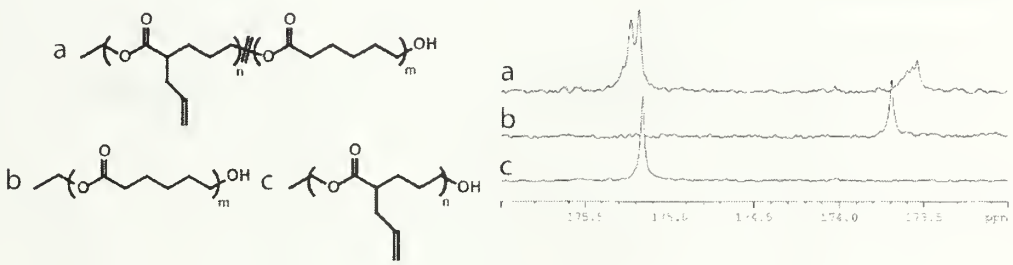


Figure 2.3 ^{13}C NMR spectra of a) copolymer $\mathbf{2_c}$ (74% 1 and 26% ε-CL), b) ε-CL homopolymer, and c) allyl-functionalized homopolymer $\mathbf{2_d}$

Incorporation of allyl functionality pendent to the aliphatic polyester backbone proved to have a substantial impact on the crystallinity of aliphatic polyesters of type **2**. Homopolymers of ε-CL with molecular weights of 6-10 x 10^3 g/mol exhibit melting transitions around 55 °C, while a copolymer with 93% ε-CL and 7% **1** melted at 35 °C. This observed melting point depression is due to disruption of polymer crystallinity by the presence of the pendent functional groups. Aliphatic polyesters with 75% ε-CL and

25% **1** melted at 8 °C, while copolymers with greater than 25% incorporation of **1** did not exhibit a melting transition. Studies on melting point depression for copolymers of δ-VL and ε-CL by Storey and Hoffman reveal a minimum melting temperature of 10 °C at 40% incorporation of δ-VL into copolymers with ε-CL.[14] The minimum was observed because the homopolymers of both ε-CL and δ-VL are semi-crystalline and have higher melting points than their copolymers as shown in Figure 2.4a. In contrast, allyl-functionalized homopolymer $\mathbf{2}_d$ is completely amorphous. Consequently melting point depression is only observed in copolymers of **1** and ε-CL with less than 25% incorporation of **1** as shown in Figure 2.4b. Copolymers with greater than 25% incorporation of **1** do not display a melting transision and are viscous liquids at room temperature.

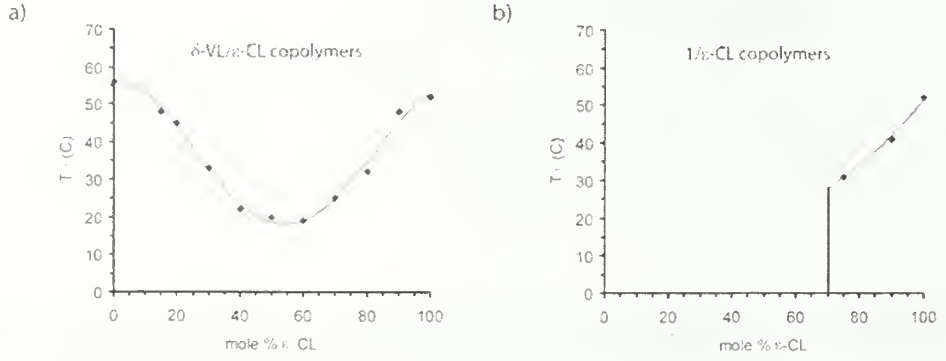


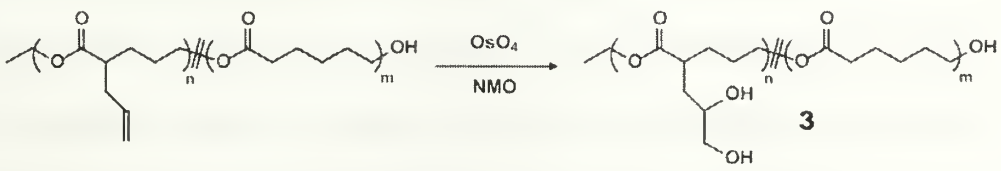
Figure 2.4 Melting point depression observed in copolymers of a) δ-VL with ε-CL, and b) allyl-VL with ε-CL

The complete disruption of crystallinity observed in aliphatic polyesters of type **2**, with greater than 25% incorporation of allyl-substituted monomer **1** represents a substantial departure from the properties of conventional aliphatic polyesters. Such properties are desirable for extending aliphatic polyesters into applications where liquid

materials are preferred or required (i.e. low temperature injection molding of degradable materials). In addition, the pendent allyl groups provide chemical handles that can be incorporated in a well-controlled fashion, and used for further chemical transformations.

2.1.3 Dihydroxylation of allyl-functionalized polyesters of type 2

The chemical transformation of olefins offers a means by which allyl-functionalized aliphatic polyesters of type **2** can be modified to alter their properties. Initial efforts focused on the conversion of the pendent allyl groups in polyesters of type **2** to 1,2-diols in order to increase hydrophilicity and provide stable hydroxyl groups for further functionalization. Olefins are readily converted to 1,2-diols using N-methylmorpholine-N-oxide (NMO) with a catalytic amount of osmium tetroxide (OsO_4).[15] These conditions are milder than conventional permanganate oxidation, or the use of a stoichiometric amount of OsO_4, and thus more suitable for use with degradable aliphatic polyesters. Treatment of allyl-substituted polyesters of type **2** with NMO (1 equiv.) and OsO_4 (1 mole percent relative to allyl functionality) in acetone gave complete conversion of the olefins to 1,2-diol groups, affording aliphatic polyesters of type **3**, as depicted in Scheme 2.1.



Scheme 2.1 Dihydroxylation of allyl-functionalized polyesters of type 2 with OsO_4/NMO to afford hydroxyl-functionalized polyesters of type 3

The diol-functionalized products were isolated by extraction into dichloromethane (CH_2Cl_2), followed by washing with water and brine, drying over $MgSO_4$, and removal of the solvent. Purification was achieved by dissolution into acetone and precipitation into cold hexanes. Successful transformation was confirmed by ^1H NMR spectroscopy, specifically the disappearance of the olefin signals of **2** at 5.75 and 5.06 ppm, and the appearance of signals at 3.65 and 3.40 ppm, corresponding to C*H*$_2$OH and C*H*(OH)CH$_2$OH respectively in **3**.

GPC analysis of pendent 1,2-diol-functionalized polyesters of type **3**, performed immediately upon isolation, revealed little change in molecular weight and polydispersity (e.g., polymer $\mathbf{2_a}$ 21% **1**, M_n 5800 g/mol, PDI 1.15 before dihydroxylation; polymer $\mathbf{3_a}$ M_n 6000 g/mol, PDI 1.13 after dihydroxylation) indicating that the desired transformation was achieved in the absence of degradation. However, periodic analysis of polyesters of type **3** that were stored under ambient conditions did show evidence of polyester degradation (Figure 2.5a). Polyesters with greater incorporation of pendent 1,2-diols were found to degrade to a greater extent than those with lower incorporation. In materials with greater than 50% 1,2-diol containing monomer units, polymer degradation occurred so rapidly that no polymer was isolated following the dihydroxylation reaction. This degradation presumably occurs by intramolecular transesterification, a cyclization reaction of the pendent primary hydroxyl group with the adjacent backbone carbonyl to form 6-membered rings as depicted in Figure 2.5b. The fact that degradation appears to level off in samples with relatively low incorporation of 1,2-diol supports this hypothesis. However, no spectroscopic data was obtained to verify this mechanism. While rapid degradation of

aliphatic polyesters will be useful for certain applications, this approach did not provide the stable, functionalized polyester-precursors desired in this thesis research for further coupling reactions.

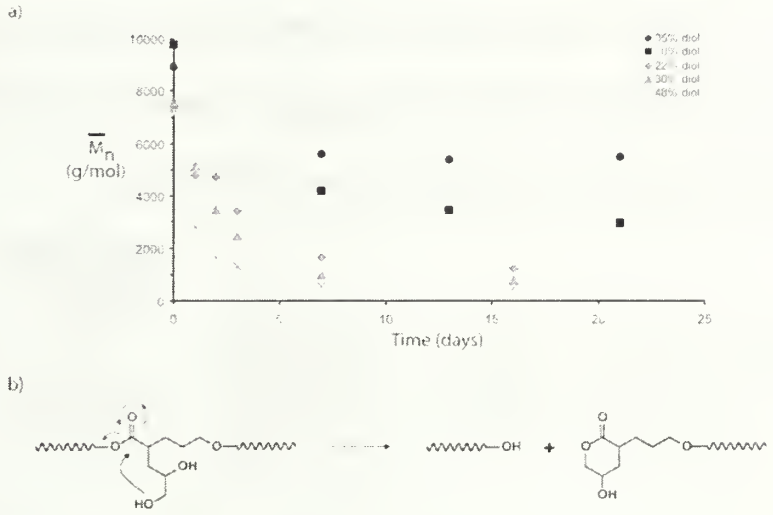


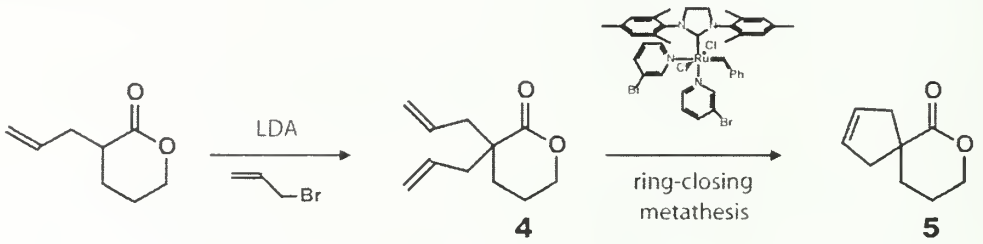
Figure 2.5 a) Degradation of polyesters of type 3 as a function of time, determined by GPC evaluation, b) proposed mechanism for degradation by intramolecular transesterification

2.2 Aliphatic polyesters with pendent cyclopentene groups

In efforts to improve the stability of hydroxyl-functionalized polyesters it was hypothesized that the introduction of a cyclic group between the hydroxyl functionality and the polyester backbone would prevent backbiting due to steric constraints. Consequently, a lactone bearing a cyclopentene group α to the carbonyl was prepared, and its polymerization chemistry was investigated. This study ultimately gave the first reported example of water-soluble PEG-grafted aliphatic polyesters with controlled molecular weights and grafting densities.[16]

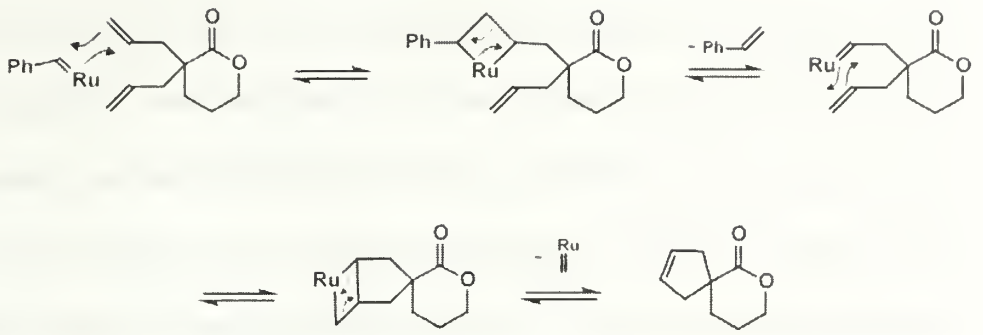
2.2.1 Synthesis of α-cyclopentene-δ-valerolactone 5

The synthesis of α-cyclopentene-δ-valerolactone (**5**) was accomplished in three steps from commercially available δ-VL. Allyl substitution of δ-VL, was performed as described previously to give lactone **1**. A second allyl substitution was then performed to give α,α-diallyl-δ-valerolactone (**4**) in 90% yield. Finally, a ring-closing metathesis was performed using Grubbs' "third-generation" ruthenium benzylidene catalyst to afford the cyclopentene-substituted product **5** (Scheme 2.2).[17]



Scheme 2.2 Synthesis of α-cyclopentene-δ-valerolactone 5

The proximity of the two allyl groups present in lactone **4** favors intramolecular ring-closing metathesis, relative to other possible reactions such as intermolecular cross-metathesis. The mechanism of ring-closing metathesis is shown in Scheme 2.3. High yields of **5** were achieved at a lactone concentration of 0.2 M in CH_2Cl_2. Initially, the ruthenium benzylidene catalyst reacts with one of the allyl groups to form a metallocyclobutane intermediate. Loss of styrene is then followed by the formation of a second metallocyclobutane by reaction with the second allyl group. Loss of ruthenium methylidene gives the desired lactone **5**, with the cyclopentene group α to the carbonyl. The catalytic cycle then continues by reaction of the ruthenium methylidene with another equivalent of **4** resulting in ring-closing and the evolution of ethylene.



Scheme 2.3 Mechanism for ring-closing metathesis of lactone 4 to yield α-cyclopentene functionalized lactone 5

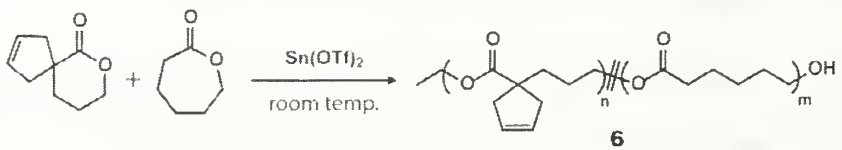
Kugelrohr distillation gave lactone **5** as a colorless, viscous liquid in 60% overall yield. Lactone **5** was characterized by high resolution mass spectrometry (EI (m/z): M^+ calcd. for $C_9H_{12}O_2$, 152. 084; found 152. 083) and NMR. The ^1H NMR spectrum of **5** revealed the disappearance of the allyl signals from lactone **4** at 5.75 and 5.11 ppm, and the appearance of a singlet at 5.61 ppm, corresponding to olefin methylene protons of the cyclopentene ring. Notable signals in the ^{13}C NMR spectrum appear at 177.2 (*C*=O), 127.6 (*C*H_2=CH_2), and at 47.7 ppm for the tetra-substituted spiro-carbon α to the carbonyl group.

2.2.2 Incorporation of cyclopentene-functionalized lactone 5 into aliphatic polyesters

Homopolymerization of lactone **5** was attempted under a variety of conditions. At elevated temperatures (110-180 °C) no polymerization was observed even after a five day reaction time. Polymerization at lower temperature (4 °C), using $Sn(OTf)_2$ as the catalyst, was slightly more successful. Initial oligomerization was observed after 2 days reaction time. Continued polymerization reached 2100 g/mol (GPC) after 14 days,

which corresponds to a degree of polymerization of 8. The ^{1}H NMR spectrum revealed a signal at 4.05 ppm, which corresponds to the methylene protons adjacent to the oxygen in the polymer (-COOCH_2-), and is shifted upfield from the corresponding lactone signal at 4.38 ppm. The relative integrations of these signals gave a monomer to polymer conversion of 12%. The improved propagation of **5** at lower temperature suggests that the ceiling temperature of the polymerization is rather low, and that in subsequent studies in the Emrick group, homopolymerization of **5** might be feasible at lower temperatures using aluminum alkoxide catalysts. In this thesis work, the focus with monomer was **5** shifted to its copolymerization with unfunctionalized lactones.

Ring-opening copolymerization of lactone **5** with ε-CL was performed at room temperature with $Sn(OTf)_2$ and EtOH as described previously. These copolymerizations reached nearly full conversion of both monomers (97% by ^{1}H NMR spectroscopy) after 48 hours to yield cyclopentene-functionalized polyesters of type **6**, as depicted in Scheme 2.4. Polyesters of type **6** were prepared with narrow polydispersities (PDI = 1.1-1.2), and controlled molecular weights over the range of 5,000-15,000 g/mol, as estimated by GPC.

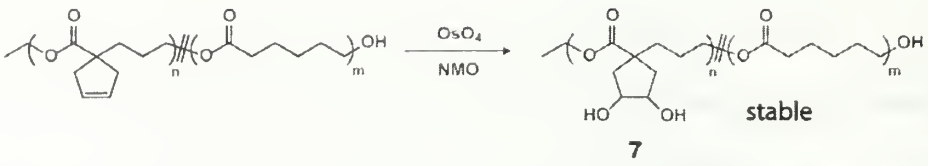


Scheme 2.4 Copolymerization of cyclopentene-functionalized lactone 5 to give polyesters of type 6

A maximum of 20% lactone **5** was incorporated into copolymers with ε-CL under the ring-opening polymerization conditions described here. The moderate incorporation of **5** is due to its low polymerizability relative to ε-CL. The presence of pendent cyclopentene groups in functionalized polyesters of type **6** was confirmed using ^{1}H NMR spectroscopy, by the presence of a doublet at 2.85 ppm corresponding to the methylene protons α to the olefin. This signal is shifted slightly up-field relative to the signal for the corresponding protons in lactone **5**. The ^{13}C NMR spectrum of polyester **6** contains carbonyl resonances at 177.3 and 173.6 ppm, which appear broadened due to the generally random incorporation of the two monomer units. Melting point depression is also observed in copolymers of type **6** up to15% incorporation of **5**. Above 15% incorporation the copolymers are completely amorpous.

2.2.3 Dihydroxylation of cyclopentene-functionalized aliphatic polyesters of type 6

The pendent olefins of cyclopentene-functionalized polyesters were converted to 1,2-diols using OsO_4 (2 mole percent) and NMO (2 equivalents relative to cyclopentene) in dimethylformamide (DMF). These reaction mixtures were stirred at room temperature for 8 h., then extracted with CH_2Cl_2, washed with brine, and filtered through a plug of activated carbon and silica gel. Removal of the solvent afforded hydroxyl-functionalized aliphatic polyesters of type **7**, as shown in Scheme 2.5. The ^{1}H NMR spectrum of the isolated products show no evidence of residual olefin signals, and a new signal centered at 3.41 ppm was observed, corresponding to the methine protons α to the hydroxyl groups.



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Scheme 2.5 Dihydroxylation of aliphatic polyesters with pendent cyclopentene functionality to give polyesters type 7

Hydroxyl-functionalized polyesters of type **7** do not undergo the rapid backbone degradation observed in the prior studies on 1,2-diols from pendent allyl-functionalized polyesters. For example polyester $\mathbf{6}_a$ (M_n 8.5 x 10^3 g/mol, PDI 1.12), gave polyester $\mathbf{7}_a$ (M_n 8.6 x 10^3 g/mol, PDI 1.16) upon hydroxylation. This polymer proved to be stable for months when stored neat under ambient conditions. The markedly enhanced shelf-stability is attributed to two factors: 1) the absence of primary alcohols as nucleophiles, and 2) the ring-strain that would be imposed by intramolecular transesterification.

While the pendent 1,2-diols in polyesters of type **7** provide increased hydrophilicity relative to unfunctionalized polyesters, it is not sufficient to impart solubility in water, EtOH, or other polar protic solvents. However, the shelf-stability of these copolymers is extremely valuable in providing functional precursors for further coupling reactions as described in the next section.

2.2.4 Poly(ethylene glycol) grafting of hydroxyl-functionalized polyesters of type 7

PEG is well known for its water solubility, amphiphilicity, and antifouling properties whereby it can resist protein adsorption. PEGylation is used to increase the residence time of hydrophobic drugs in the bloodstream by improving solubility in water and preventing rapid protein adsorption, which results in recognition and elimination of foreign materials by macrophages.[18-20] Water-soluble aliphatic polyesters

are highly desirable for biomaterial applications, particularly in cases where intravenous injections are desired, such that the material will circulate in the blood stream. Such polyesters are typically prepared by chain-end functionalization and prior to this thesis work, there were no reports on the controlled synthesis of PEG-grafted aliphatic polyesters with sufficient grafting to impart water solubility. In contrast to chain-end functionalization, pendent functionalization should provide homogeneity of the material properties during degradation, and offer multiple opportunities for integration of diverse functional groups with controlled density or "loading".

Grafting of PEG chains pendent to aliphatic polyesters was accomplished by dicyclohexylcarbodiimide (DCC) mediated coupling of hydroxyl-functionalized polyesters of type **7** to succinic acid-functionalized derivatives of PEG monomethyl ethers (**8**) as shown in Figure 2.6.

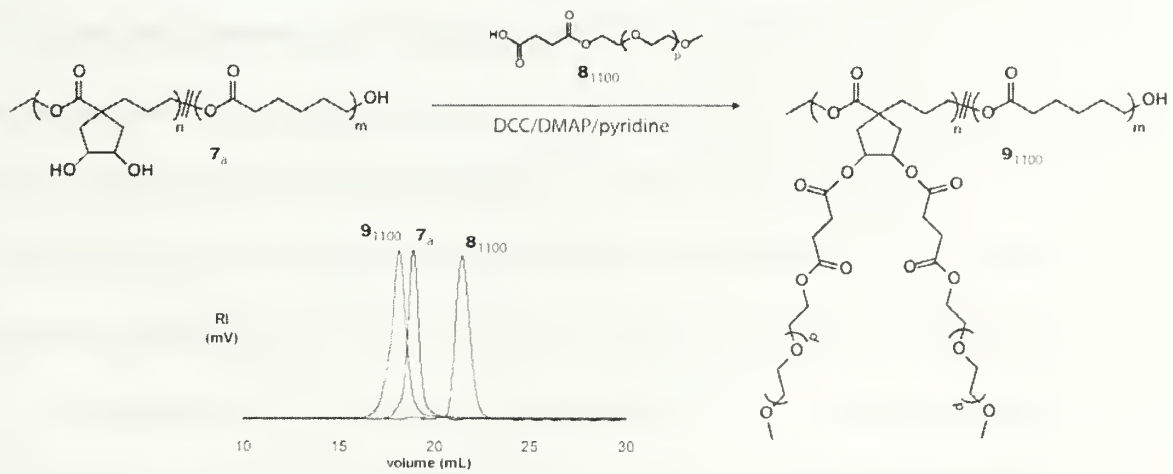


Figure 2.6 DCC coupling of succinic acid-functionalized PEG with hydroxyl-functionalized polyesters including GPC traces of 1) hydroxyl-functionalized polyester 7a, 2) succinic acid-functionalized PEG 8_{1100}, and 3) PEG-grafted polyester 9_{1100}

The molecular weights of the PEG chains used in these studies were 164 (triethylene glycol monomethyl ether) ($\mathbf{8}_{TEG}$), 750 ($\mathbf{8}_{750}$), and 1100 ($\mathbf{8}_{1100}$) g/mol. Triethylene glycol monomethyl ether was distilled prior to use, while PEG-750 and PEG-1100 monomethyl ethers, purchased from Fluka, required purification by column chromatography on silica gel to remove PEG-diol impurities. Carbodiimide coupling of PEG-acid **8** and polyester $\mathbf{7}_a$ was performed in refluxing CH_2Cl_2 for 24 hours. The resulting graft-copolymer products were purified by dialysis in 95% ethanol (Spectra/Por® Membrane MWCO 6-8000) to remove unreacted **8**. Precipitation of a concentrated acetone solution of the product into cold hexanes followed by filtration and drying under reduced pressure gave PEG-grafted polyesters of type **9**. GPC traces of hydroxyl-functionalized polyester $\mathbf{7}_a$, succinic acid-functionalized PEG monomethyl ether $\mathbf{8}_{1100}$, and PEG-grafted product $\mathbf{9}_{1100}$ are shown in Figure 2.6. The narrow molecular weight distribution of PEGylated polymer $\mathbf{9}_{1100}$ demonstrates that the PEG-grafting chemistry can be performed without cross-linking or backbone degradation.

The number of PEG grafts per polymer backbone shown in Table 2.2, was estimated from ^{1}H NMR spectra, by the relative integration of the signals at 4.01 (-C*H*$_2$OCO polyester) and 4.34 ppm (-C*H*$_2$OCO PEG). 14-16 PEG grafts per polyester chain or 1 graft per every 5 repeat units was achieved starting from hydroxyl-functionalized polyester $\mathbf{7}_a$ (28 mole percent pendent hydroxyl groups relative to unfunctionalized monomer units). This corresponds to a grafting efficiency of 50-58%, suggesting that the grafting of one PEG-chain to the cyclopentyl group inhibits the grafting of a second PEG-chain due to steric interference.

Table 2.2 Characterization of PEG-grafted polyesters type 9 and their precursors

Polymer	M_n x 10^3 g/mol[a]	PDI[a]	# PEG grafts[b]	water solubility
$\mathbf{6}_a$	8.5	1.12	NA	minimal
$\mathbf{7}_a$	8.6	1.16	NA	minimal
$\mathbf{9}_{TEG}$	11.7	1.20	16	minimal
$\mathbf{9}_{750}$	15.5	1.21	14	stable dispersion
$\mathbf{9}_{1100}$	16.5	1.14	14	excellent

[a] determined by GPC relative to polystyrene standards; [b] determined from 1H NMR spectra

Triethylene glycol monomethyl ether substitution of copolymer $\mathbf{7}_a$ to give copolymer $\mathbf{9}_{TEG}$, using TEG-acid $\mathbf{8}_{TEG}$, gave a graft copolymer which was soluble in organics e.g. acetone, CH_2Cl_2, and THF, but not water or methanol (MeOH). Copolymer $\mathbf{9}_{750}$ with 750 g/mol PEG grafts was soluble in organics and MeOH, and formed stable dispersions in water. Copolymer $\mathbf{9}_{1100}$ with 1100 g/mol PEG grafts was completely water-soluble at concentrations in excess of 250 mg/mL in addition to being soluble in many common organic solvents.

2.2.5 Future outlook for functionalization of polyesters with pendent diols

The potential use of stable 1,2-diol functionalized aliphatic polyesters is not limited to the PEGylation chemistry described in this chapter. Carbodiimide coupling could be employed to incorporate numerous carboxylic acid-functionalized moieties including organic dyes and drugs for degradation and release studies. In addition to carbodiimide couplings pendent hydroxyl-functionalized polyesters of type 7 should also allow for coupling of pendent phospholipid moieties, as depicted in Figure 2.7, by reaction with 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by trimethylamine. Such moieties

have been shown to greatly improve blood compatibility and could have a dramatic impact on the application of aliphatic polyesters.[21-22]

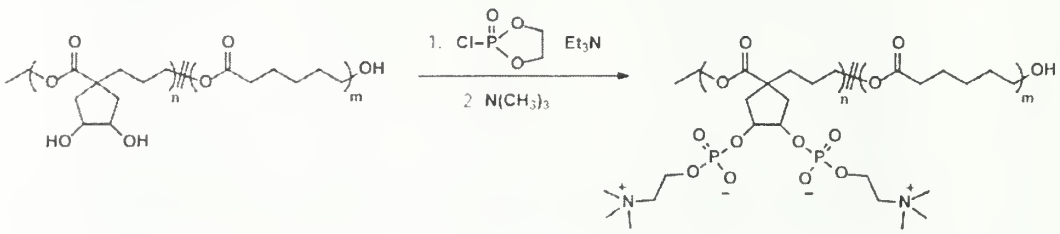


Figure 2.7 Grafting of phospholipid moieties to hydroxyl-functionalized polyesters of type 7

2.3 Synthesis of bis-δ-valerolactone and cross-linking of aliphatic polyesters

Linear aliphatic polyesters are desirable materials for tissue engineering scaffolds, localized drug delivery depots, and various other kinds of implanted devices because of their controlled degradation behavior. Devices composed of such polyesters suffer from a lack of structural stability upon degradation and thus the polyester is often cross-linked such that the device maintains its origin dimensions until the latest stages of degradation.[23] Cross-linking can be achieved by several methods including the use of peroxide, electron beam treatment, and block copolymerization of crystalline and amorphous segments which form physical cross-links. However, the crystalline cross-link regions of these thermoplastic elastomer networks degrade much slower than the amorphous regions, which disrupts the normally reproducible nature of polyester degradation. It is desirable therefore to introduce polyester cross-linking during ring-opening polymerization using a bis-lactone such that the network, including the cross-links, is composed entirely of amorphous aliphatic polyester. Albertsson and coworkers reported the synthesis of (2,2)-bis(ε-caprolactone-4-yl) propane (BCP) and cross-

linking of 1,5-dioxepan-2-one during ring-opening polymerization as depicted in Figure 2.8.[24] BCP was also employed in the preparation of biodegradable elastomers by Amsden and coworkers who used BCP to cross-link star copolymers of lactide and ε-CL.[23]

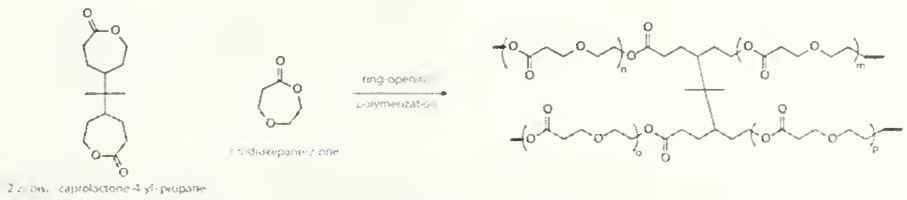
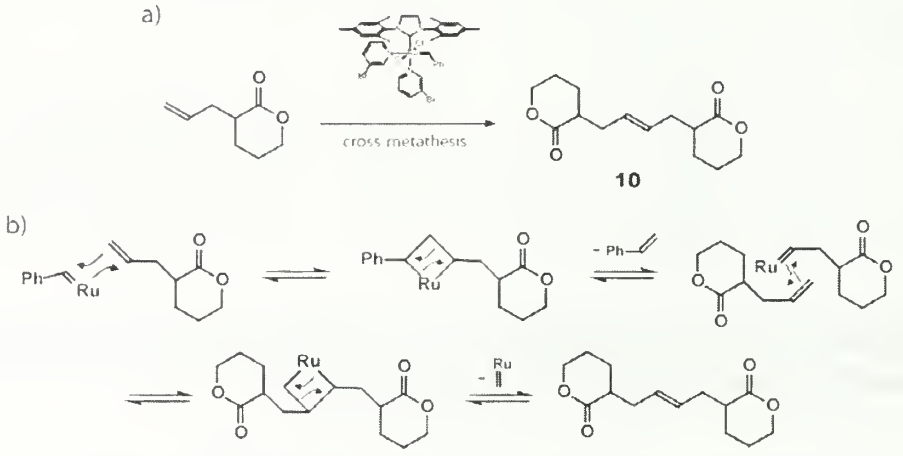


Figure 2.8 (2,2)-bis-ε-caprolacatone-4-yl) propane and cross-linking during ring-opening polymerization of 1,5-dioxepane-2-one

In the course of metathesis studies conducted during this thesis work a bis-lactone,1,4-bis(δ-valerolactone-yl)-2,3-butene (**10**), was synthesized as depicted in Scheme 2.6a. Bis-lactone **10** was prepared by cross-metathesis of allyl-functionalized lactone **2** using Grubbs' third generation ruthenium benzylidene catalyst. The lactone and catalyst were dissolved in CH_2Cl_2 (1 M) and stirred at room temperature for 6 h. The solvent was then removed and the crude product purified by column chromatography eluting with 20% EtOAc/hexane to afford bis-lactone **10** as a white crystalline solid in 67% yield. In the first step of the cross-metathesis mechanism, shown in Scheme 2.6b, the ruthenium benzylidene catalyst reacts with allyl-functionalized lactone **2** to form a metallocyclobutane intermediate. Styrene is then eliminated and a ruthenium allyl complex is formed. This complex reacts with a second equivalent of **2** to form a second metallocyclobutane intermediate. Upon loss of ruthenium methylidene, the desired bis-lactone **10** is formed and the catalytic cycle is continued by further reaction of ruthenium methylidene with **2** to give the bis-lactone

and ethylene as a side product. Both *cis* and *trans* substitution of the double bond of bis-lactone **10** should result from the cross-metathesis.



Scheme 2.6 a) Synthesis of 1,4-bis(δ-valerolactone-yl)-2,3-butene 10, b) mechanism for ring-closing metathesis

The structure of bis-lactone **10** was confirmed in the ^{1}H NMR spectrum by the precense of signals at 5.51 and 4.40 ppm corresponding to the olefin methylene protons (C*H*=C*H*) and the lactone methylene protons adjacent to the oxygen (COOCH_2) respectively. The relative integrations of these signals give a ratio of 1 olefin per 2 lactones indicative of the bis-lactone structure. The ^{13}C NMR spectrum displays resonances for 7 carbons at 174.35 (*C*OOCH$_2$), 130.16 (*C*H=*C*H), 69.03 (COO*C*H$_2$), 40.14 (*C*HCOO), 34.69 (*C*H$_2$CH=CH), 24.65 (*C*H$_2$CH$_2$CH$_2$O), and 22.47 (*C*H$_2$CH$_2$CH$_2$O) ppm.

Cross-linking of aliphatic polyesters using bis-lactone **10** was demonstrated by running two ring-opening polymerizations of ε-CL side by side, one with only ε-CL and one with 10 mole percent bis-lactone **10** added. Both polymerizations were initiated with EtOH and catalyzed by 2 mole percent Sn(OTf)$_2$. After stirring for 24 h. at room

temperature the polymerization that did not contain bis-lactone **10** was dissolved in acetone and precipitated into cold hexanes. GPC analysis of the resulting polymer gave Mn 6,200 g/mol and PDI 1.14. In contrast the polymerization that contained 10 mole percent bis-lactone **10**, gelled after 8 h. and could not be dissolved for further analysis. After 24 h. the gel was physically broken up and small pieces were shown to swell in acetone, THF, and CH_2Cl_2. Such materials could prove particularly valuable for low temperature injection molding of fully degradable implantable devices. Additionally, the residual unsaturations in the cross-links could be used for further chemical transformations.

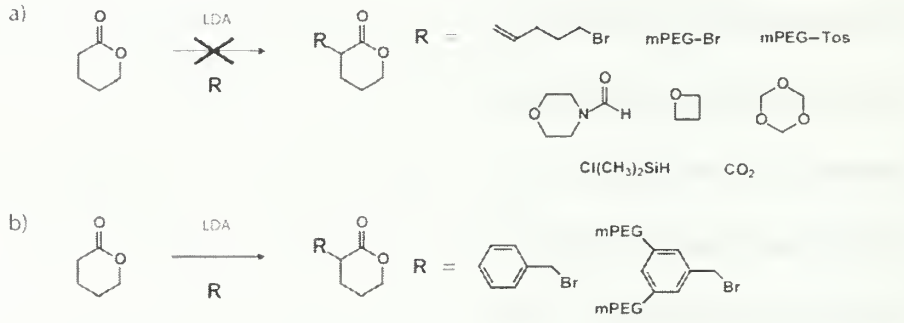
2.4 Additional attempted synthesis

The scope of aliphatic polyester functionalization will always be limited by the degradable nature of the polyester backbone. While the focus of this thesis work is on successful functionalization, it is important to briefly discuss the attempted functionalizations that were found to be incompatible with controlled aliphatic polyester synthesis due to difficulties encountered in the monomer synthesis, insufficient polymerizability of hindered lactones, and/or degradation and cross-linking encountered during post-polymerization modifications.

2.4.1 Attempted substitutions of δ-valerolactone

Based on the success of substitution of δ-VL at the α position with allyl functional groups, a number of other electrophiles were employed in related synthetic attempts. The procedure for each of these attempted substitutions was similar to that for the synthesis of lactone **1**, with the exception that allyl-bromide was replaced by the

corresponding electrophile. Scheme 2.7 depicts a) attempted substitutions in which the product was not achieved, and b) substitutions in which the product was prepared, but subsequently found not to polymerize.



Scheme 2.7 Attempted substitution of δ-valerolactone with various electrophiles a) unsuccessful substitutions, b) successful substitutions, but lack of polymerizability

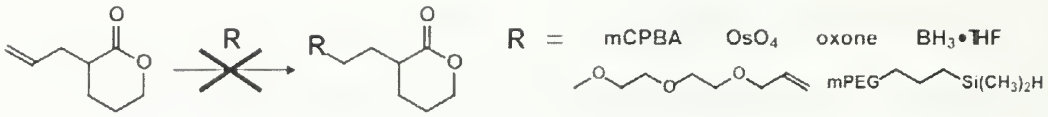
To investigate the effect of a carbon spacer between the polyester backbone and pendent functional groups, lactone substitution with 5-bromopentene was attempted. Polymerization of such a lactone and subsequent dihydroxylation would give polyesters with pendent 1,2-diols that would form 8-membered rings upon intramolecular cyclization. As the formation of 8-membered rings is much less probable, this system could provide further evidence into the mechanism of the degradation observed for the 1,2-diol-functionalized polyesters derived from an allyl-substituted lactone described in Section 2.1.3. Several attempts at the substitution were made varying the concentration and the temperature with no evidence of product formation. Similarly, substitution of δ-VL with PEG chains was attempted using PEG-bromide and PEG-tosylate derivatives with no product formation observed. Lactones with hydroxyl substituents can be used to prepare hyperbranched polyesters as the hydroxyl group initiates lactone polymerization and thus the monomer and initiator are contained in the same molecule.

Toward this end, substitution of δ-VL with dioxane and trioxane was attempted, but not achieved. The synthesis of lactone monomers with aldehyde, carboxylic acid, and silane functionalities were also investigated by quenching the enolate of δ-VL with N-formylmorpholine, carbon dioxide, and chlorodimethylsilane respectively. In all cases shown in Scheme 2.7a unsubstituted δ-VL was recovered as the only product. Presumably the quenching agents are not reactive enough electrophiles to effect the substitution at the reduced temperatures required to minimize lactone ring-opening (-78 - -40 °C).

On the other hand, lactone substitution with phenyl bromide and TEG-derivatized phenyl bromide, as depicted in Scheme 2.7b, was successful. In the case of phenyl substitution, the resulting lactone homo-/copolymerized under $Sn(OTf)_2$ mediated conditions. However, the TEG-derivate could not be homo-/copolymerized despite attempts at elevated temperature with $Sn(Oct)_2$.

2.4.2 Attempted modifications of allyl-functionalized δ-valerolactone 1

Several attempts were made to modify allyl-functionalized δ-valerolactone **1** as depicted in Scheme 2.8. Epoxidation with either m-chloroperoxybenzoic acid (mCPBA) or oxone to give an epoxide ring in the α-position that could be useful in further transformations resulted in mixtures of products that were not able to be separated by extractions, column chromatography, and/or distillation. Attempts to prepare lactones with hydroxyl substituents by oxidation of the allyl group with either osmium tetroxide or BH_3·THF followed by oxidative work-up did not give the desired products and no intact lactone was observed.

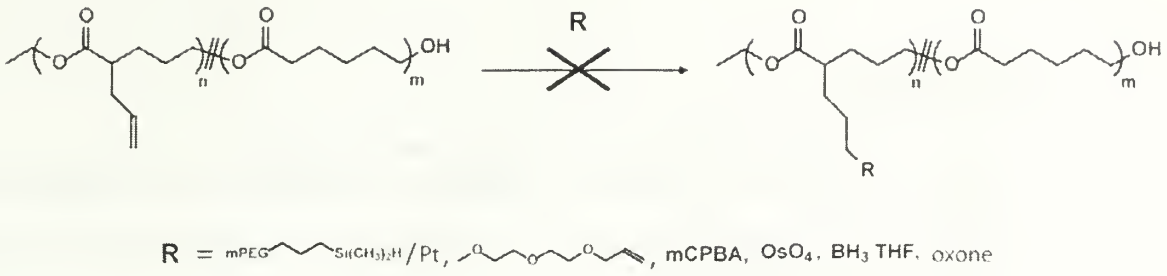


Scheme 2.8 Attempted modifications of allyl-functionalized lactone 1

Hydrosilation with PEG derivatized chlorodimethylsilane and Karstendts' catalyst was successful, but the purity obtained was insufficient to allow polymerization. PEG-functionalization was also attempted by cross-metathesis with allyloxy-terminated PEG monomethyl ethers using Grubbs' third-generation ruthenium benzylidene catalyst. However, regardless of the concentration and relative molar excess of allyloxy-PEG employed, the bis-lactone product **10**, described in Section 2.3, was formed as the major product.

2.4.3 Attempted post-polymerization modifications of allyl-functionalized copolymers of type 2

Several post-polymerization modifications of allyl-functionalized polyesters, depicted in Scheme 2.9, were also attempted with little success. Epoxidation with mCPBA was successful as determined by the disappearance of the allyl signals at 5.75 (CH=CH_2) and 5.06 (CH=CH_2) ppm, in the ^{1}H NMR spectrum, and the appearance of signals at 2.90, 2.75, and 2.47 ppm corresponding to the three protons on the epoxide ring. Purification by extraction, precipitation, and column chromatography, however, were not successful in isolating the desired product from residual *m*-chloroperoxybenzoic acid formed during the reaction. Epoxidation attempted with oxone resulted in degradation of the polyester backbone.



Scheme 2.9 Attempted post-polymerization modifications of allyl-functionalized polyesters

Transformation of the pendent allyl functionality to hydroxyl groups using either OsO_4/NMO or BH_3·THF followed by oxidative work-up also resulted in degradation of the polyester backbone (described in detail for OsO_4/NMO oxidation in Section 2.1.3) Attempted cross-metathesis of the pendent allyl groups with allyloxy-terminated PEG produced no reaction and only starting materials were recovered. In contrast, PEG-grafting by hydrosilation with PEG-derivatized chlorodimethylsilane was more successful. The PEG-silane was synthesized by initial hydrosilation of allyloxy-terminated PEG monomethyl ether, followed by reduction of the chloride with lithium aluminum hydride (synthetic details found in Chapter 5). A second hydrosilation, performed on the polyester, was attempted under a variety of conditions utilizing both Karstedts' catalyst and H_2PtCl_6 as the Pt source. In some cases clean products were obtained with no evidence of degradation or cross-linking, but the results were not entirely reproducible (in many cases cross-linking was observed due to an unknown side reaction

2.3 References

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CHAPTER 3

POLYESTERS WITH PENDENT ALKYNES

The alkene-functionalized polyesters described in Chapter 2 afforded the first reported water-soluble PEG-grafted polyesters with controlled molecular weights and polydispersities. However, the methodology employed gave copolymers with a maximum 20% incorporation of cyclopentene-functionalized lactone **5**, which inherently limits the extent of PEGylation or other types of functionalization to rather moderate levels. Efforts thus continued towards the synthesis of a functionalized lactone monomer that could be homopolymerized, and modified cleanly following polymerization, to give higher degrees of pendent functionalization. Described in this chapter is the synthesis of a lactone monomer with an alkyne group α to the carbonyl, its polymerization to give aliphatic polyesters with pendent alkynes, and post-polymerization chemistry on these alkynes. Particular emphasis is placed on functionalization of the alkyne-substituted polyesters using "click" chemistry, specifically the Cu(I) catalyzed cycloaddition of alkynes and azides.

3.1 Introduction to click chemistry

"Click" chemistry, recently coined by Sharpless, Finn, and Kolb, refers to a set of highly efficient cycloaddition reactions that can be carried out cleanly and regiospecifically in the absence of side reactions.[1] The stepwise Cu(I)-catalyzed analog of the Huisgen 1,3-dipolar cycloaddition of azides and acetylenes is the most widely employed click reaction and forms the basis of this work.[2] Click chemistry of this type also benefits from the facile introduction of azide and acetylene groups into organic and

polymer molecules, the stability of these groups to many other reaction conditions, and the tolerance of the reaction to the presence of other functional groups. Consequently, such chemistry can be employed in a modular sense as depicted in Figure 3.1.

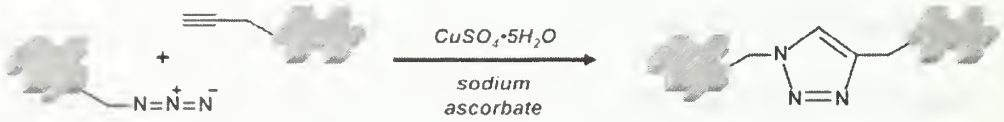


Figure 3.1 Cylcoaddition of acetylenes and azides using a type of click chemistry catalyzed by copper(I)

The cycloaddition of azides and acetylenes to give triazoles has an extensive history and was first studied in detail by Huisgen in the 1960's.[3-4] However, in the absence of a Cu(I) catalyst, the reaction is not as efficient, and affords a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazole regioisomers depending on the substituents. For example, the cycloaddition of phenylazide and phenylacetylene gives 52% 1,5-substitution, and 43% 1,4-substitution, as shown in Figure 3.2a.

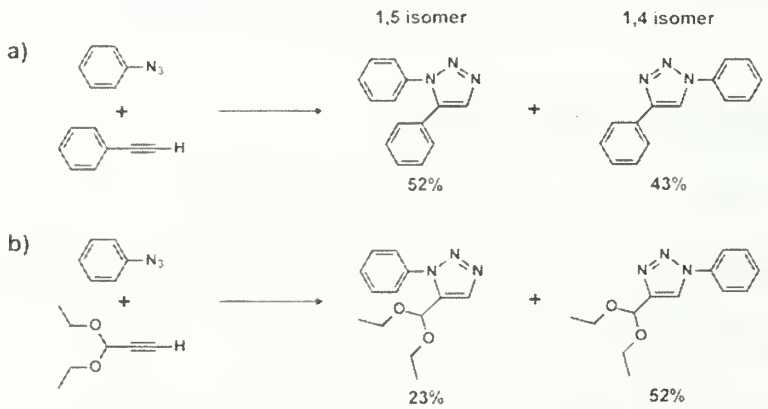
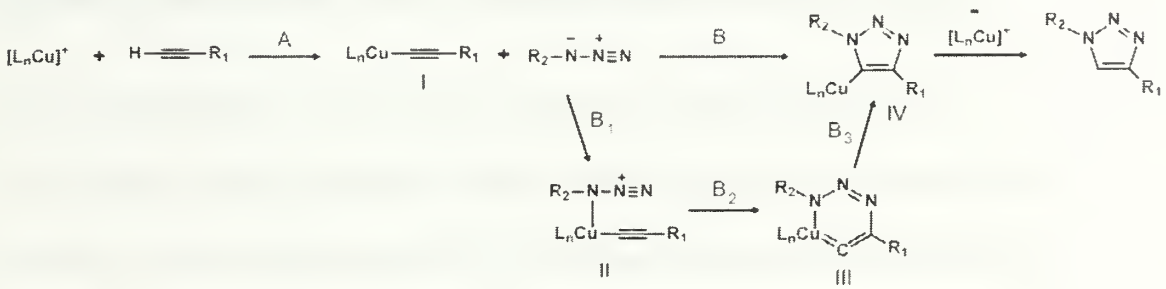


Figure 3.2 Ratios of 1,5- and 1,4-disubstituted triazole regioisomers resulting from the cycloaddition of a) phenylazide with phenyacetyelene, and b) phenylazide with propargylaldehyde acetal

In contrast, cycloaddition of phenylazide with propargylaldehyde acetal affords 23% 1,5-substitution, and 52% 1,4-substitution, as shown in Figure 3.1b. The ratio of isomers obtained depends on competing steric and electronic factors.

When the Cu(I) catalyzed stepwise analog of the Huisgen cycloaddition, which yields only the 1,4-regioisomer, was reported by Fokin and Sharpless in 2002, the efficiency and selectivity of the reaction were deemed sufficient to include it in the family of click reactions. Fokin and Sharpless describe the modified Cu(I) catalyzed reaction as a stepwise ligation of azides and acetylenes rather than a concerted cycloaddition. They propose that density functional theory calculations strongly favor the stepwise mechanism (B1→ B2→ B3 Scheme 3.1) over the concerted cycloaddition mechanism (B Scheme 3.1).



Scheme 3.1 Stepwise mechanism ($B_1 \rightarrow B_2 \rightarrow B_3$) for the Cu(I) catalyzed cycloaddition of acetylenes and azides proposed by Fokin and Sharpless

The click reaction between azides and acetylenes has been utilized elegantly in recent years by Sharpless and Finn in small molecule organic synthesis, [1-2] and by Fokin, Fréchet, and Hawker in the synthesis of dendrimers [5] (Figure 3.3a) and dendronized polymers [6] (Figure 3.3b). The mild reaction conditions and functional group tolerance of this chemistry is instrumental in its application to label biologically-

derived macromolecular structures as reported by Sharpless, Finn, and Tirrell,[7-8] and depicted in Figure 3.3c-d.

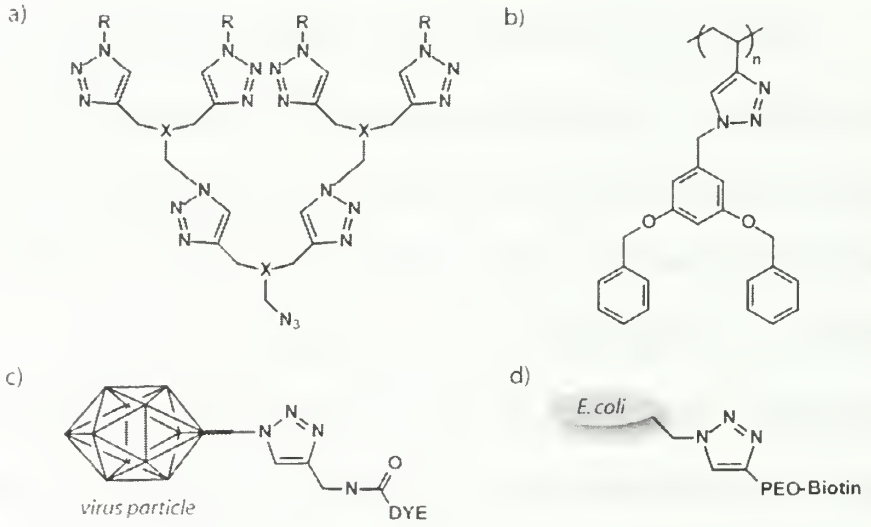


Figure 3.3 Use of click chemistry in a) dendrimer synthesis, b) dendronized polymer synthesis, and the labeling of c) virus particles, and d) bacteria

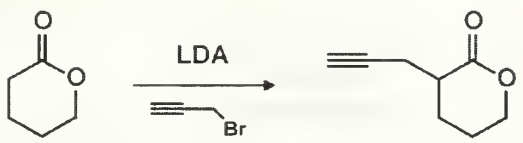
The well-demonstrated versatility and mild reaction conditions required for click chemistry made its application to aliphatic polyesters appear promising and particularly valuable given the sensitivity of aliphatic polyesters to the conditions required for many conventional organic transformations and couplings. This chapter focuses on the incorporation of acetylene groups pendent to aliphatic polyesters, and subsequent click chemistry to afford the first example of linear polyesters with high PEG-grafting density, and narrow molecular weight distribution. Click chemistry for the covalent attachment of oligopeptide sequences to aliphatic polyesters is also demonstrated.

3.2 Preparation of acetylene-functionalized aliphatic polyesters

In order to utilize click chemistry for post-polymerization modifications, an azide or acetylene group must first be incorporated into a lactone monomer. While, acetylene substitution of lactones α to the carbonyl group had been reported previously, polymerization of such lactones had not.[9] Thus, the studies presently described were initiated on the idea that acetylene-functionalized lactones would be amenable to ring-opening polymerization, and the expectation that the mild nature of click chemistry would be compatible with the aliphatic polyester backbone.

3.2.1 Synthesis of acetylene-functionalized lactones

α-Propargyl-δ-valerolactone (**11**) was prepared as described previously for α-allyl-functionalized lactone **1**, but substituting propargyl bromide for allyl bromide as depicted in Scheme 3.2. Freshly distilled δ-VL was deprotonated with LDA in THF at -78 °C, followed by quenching with a toluene solution of propargyl bromide and HMPA. Purification of the crude product by column chromatography on silica gel (0-30% EtOAc in hexanes) was followed by Kugelrohr distillation to give lactone **11** as a colorless, viscous liquid in 74% yield.



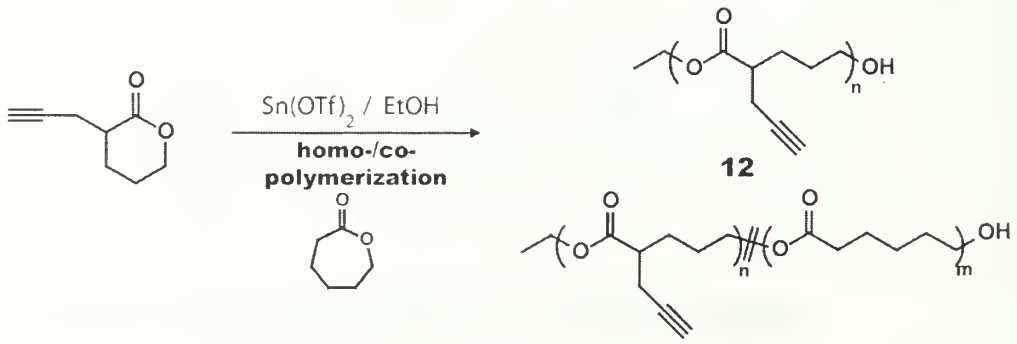
11

Scheme 3.2 Synthesis of α-propargyl-δ-valerolactone (11)

Successful acetylene substitution was confirmed by ^{1}H NMR spectroscopy in which the terminal acetylene proton of **11** is observed at 1.97 ppm. A signal at 4.28 ppm corresponds to the methylene protons δ to the carbonyl group (-C*H*$_2$O-), characteristic of the intact lactone (this methylene signal shifts upfield by ~0.2 ppm upon ring-opening). In the ^{13}C NMR spectrum of **11**, the acetylene carbons resonate at 81.1 (-*C*≡C-H) and 68.6 (-C≡*C*-H) ppm. The IR spectrum of **11** reveals an acetylene C-H stretch at 3280 cm^{-1}, and high resolution mass spectrometry (HRMS) gives a molecular mass of 139.078 g/mol (calculated 139.076 g/mol for $[M+H]^+$). As lower yields (<50%) were achieved for alkyne-functionalization of ε-CL, polymerization and post-polymerization coupling efforts focused on lactone **11**.

3.2.2 Polymerization of acetylene-functionalized lactone 11

Ring-opening polymerizations using lactone **11** were performed at room temperature; catalyzed by Sn(OTf)$_2$ and initiated by EtOH. Lactone **11** was found to homopolymerize readily, and copolymerize with ε-CL over the entire range of feed ratios, to afford pendent acetylene-functionalized aliphatic polyesters of type **12** as depicted in Scheme 3.3.[10]



Scheme 3.3 Homo-/copolymerization of acetylene-functionalized lactone 11 to afford novel aliphatic polyesters with pendent acetylenes

Lactone **11** was found to polymerize more slowly than δ-VL and allyl-substituted lactone **1**. Copolymerizations using 5 mole percent catalyst relative to the initiator, with 25% incorporation of **11**, reached >90% conversion within 36 hours. Homopolymerization of **11**, also with 5 mole percent catalyst, required 48 hours to reach this level of conversion. In contrast, homo/co-polymerizations of δ-VL and lactone **1** were complete within 24 hours at similar catalyst loading.

The increase in polyester molecular weight as a function of lactone conversion was monitored by GPC (THF) and ^{1}H NMR spectroscopic analysis of aliquots removed from the reaction mixture. Conversion was calculated by integration of the signals at 4.28 ppm (C*H*$_2$O monomer) and 4.03 ppm (C*H*$_2$O polymer backbone). The controlled nature of the homopolymerization of lactone **11** is demonstrated by the linear increase in molecular weight with conversion, and narrow polydispersities, of the isolated products show in Figure 3.4.

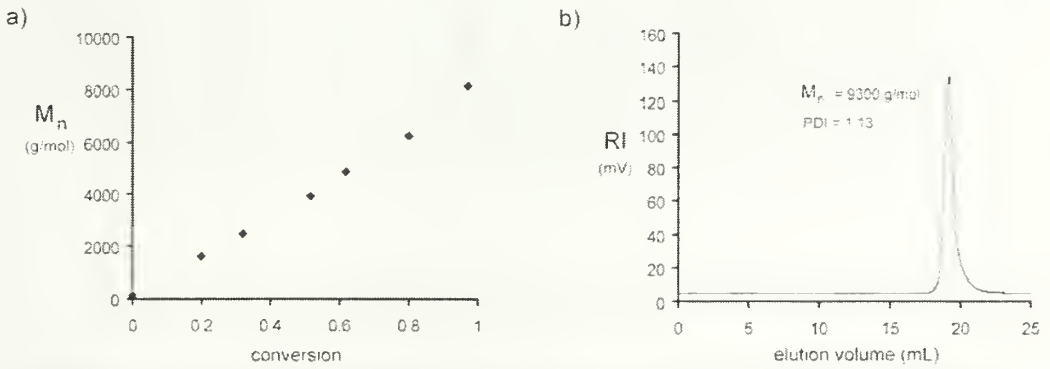


Figure 3.4 Homopolymerization of lactone 11 a) plot of molecular weight *vs.* conversion, b) GPC trace in THF of isolated product

Such control is critically important for obtaining functionalized aliphatic polyesters that can be compared and contrasted to conventional unfunctionalized versions. Moreover,

integration of this useful functionality into copolymers in controlled percentages carries the ability to impact every application area where aliphatic polyesters are used currently.

Molecular weights in the 6-15 K range were typically achieved for homo- and copolymerization, and target molecular weights were in good agreement with the monomer-to-initiator ratio. The extent of lactone **11** incorporated into the copolymer structure was calculated by integration of the ^{1}H NMR spectral signals at 2.01 ppm (C≡C-*H* from monomer **11**) against the signal at 4.03 ppm (CH_2O of the polymer backbone from **11** and ε-CL repeat units). The spectroscopically calculated incorporations of monomer **11** agree well with the feed ratios as shown in Table 3.1.

Table 3.1 Characterization of polymers with pendent acetylene

Polymer	Feed Ratio (11 : ε-CL)	Incorporation (11)[a]	M_n x 10^3 g/mol[b]	PDI[b]
12_a	10 : 90	10	7.9	1.11
12_b	25 : 75	23	8.0	1.10
12_c	50 : 50	47	7.5	1.11
12_d	100 : 0	100	6.0	1.11

[a] determined from ^{1}H NMR spectra, [b] determined by GPC relative to polystyrene standards

The agreement between feed ratios and experimentally observed monomer incorporations, along with the narrow polydispersities, demonstrates that aliphatic polyesters with pendent acetylene functionality can prepared in a well-controlled fashion.

^{1}H NMR analysis of aliquots taken from the polymerization mixture as a function of time revealed a polymer composition in close accord with the feed ratio throughout the course of polymerization. This suggests that the slower rate of polymerization for lactone **11** is due to a reversible ligand interaction between the acetylene groups and the catalyst and not a reluctance of lactone **11** to polymerize. If ring-opening of lactone **11** occurred more slowly than ring-opening of ε-CL aliquots taken at early stages of copolymerization would be rich in ε-CL. The ^{13}C NMR spectra of these copolymers exhibit several overlapping signals in the carbonyl region (from 174.1 to 173.3 ppm), indicating a random distribution of monomer units, rather than a blocky distribution expected if ring-opening of **11** was significantly slower than ε-CL.[11-12] As found in the allyl case, incorporation of lactone **11** disrupts the polyester crystallinity, which results in melting point depression at low incorporation of **11**. Polyesters with greater than 25 mole percent incorporation of **11** are amorphous.

It should be noted, that in contrast to the methods described previously for alkene-functionalized polyesters, these acetylene-functionalized materials do not require further transformation prior to productive coupling chemistries, as demonstrated in the next section. This feature adds to the simplicity and generality of the approach, and is expected to lead to a broader application base of these polyesters relative to the alkene-functionalized versions.

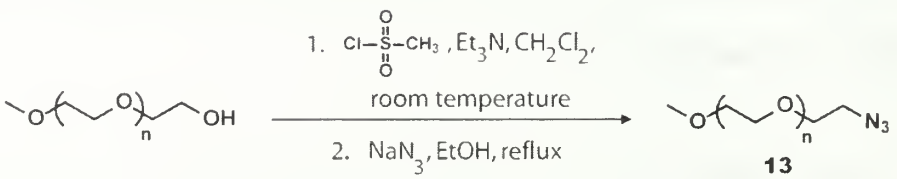
3.3 PEG grafting of acetylene functionalized polyesters by click chemistry

PEG grafting of acetylene-functionalized polyesters by click chemistry has several advantages over the cyclopentene based PEGylation method described in

Chapter 2. The most apparent are the fewer steps required to synthesize the PEG-grafted polyesters, and the fact that acetylene-functionalized lactone **11** can be both homo- and copolymerized, giving access to the entire range of functional monomer incorporation. Click chemistry is appealing from the standpoint of high product yields, and the absence of byproducts. Thus, while the cyclopentene-based approach afforded the first controlled synthesis of water-soluble, PEG-grafted polyesters, the acetylene-based method is superior with regards to grafting density and synthetic simplicity. Indeed, the impact of click chemistry on aliphatic polyester functionalization is projected to extend far beyond that described in this thesis.

3.3.1 Synthesis of PEG-grafted polyesters by click chemistry

α,ω-PEG-1100-monomethyl ether azide (**13**) was prepared for PEG grafting to acetylene-functionalized aliphatic polyesters of type **12**, following a literature procedure for the preparation mono-azide functionalized triethylene glycol, but using a 1.5 equivalent excess of reagents.[13] Mesylation of PEG-1100 monomethyl ether was performed in CH_2Cl_2, followed by nucleophilic substitution with sodium azide as shown in Scheme 3.4.



Scheme 3.4 Synthesis of azide-terminated PEG-monomethyl ether 13

The presence of the terminal azide of **13** was confirmed by observation of the characteristic azide frequency at 2105 cm^{-1} in the FTIR spectrum, and a resonance at 50.9 ppm in the ^{13}C NMR spectrum corresponding to the methylene group adjacent to the azide.

The Cu(I) catalyzed reaction of azides and acetylenes is most effective when performed in water, or mixtures of water and polar solvents such as *t*-butanol. In many cases, the reaction proceeds readily despite low aqueous solubility of one or both of the reactants.[1] Elevated temperatures (ca 50 °C) have also been employed to increase solubility and reactivity. While a variety of Cu(I) salts, such as copper(I) iodide, are used to catalyze the click reaction of azides and acetylenes, the *in-situ* formation of Cu(I) by reduction of copper(II) sulfate with sodium ascorbate is a common and synthetically simple catalyst system frequently employed.

Conditions utilized in this work deviated from "standard" click procedures in that the water insoluble acetylene-functionalized **12** was added as a concentrated solution in acetone (200 mg/mL) to a vigorously stirred solution of azide-functionalized **13** in water at elevated temperatures (~80 °C). The use of acetone and the elevated temperature gave a good dispersion of the reaction components as 80 °C is above the melting temperature of all acetylene-functionalized polyesters. Reactions performed at room temperature, or without the use of acetone, were heterogeneous, and polymer adhesion to the reaction vessel was observed. These reactions did not proceed well, giving only low grafting densities. Introduction of **12** was followed by the addition of sodium ascorbate and copper (II) sulfate, and the reaction mixture was stirred for 10-12 hours at 80 °C. The acetone evaporated during this time period, and the polyester

became soluble in the aqueous environment as the click reaction proceeded. PEG-grafted polyesters **14**, with 1,4-disubstituted-1,2,3-triazole linkages, as depicted in Figure 3.5a, were isolated by extraction into CH_2Cl_2, and purified by dialysis in phosphate buffered saline (PBS) followed by precipitation into cold hexanes. Drying under vacuum gave pure polyester-*graft*-PEG copolymers of type **14** as white semi-crystalline powders. The crystallinity in these materials is due to the PEG grafts as evidenced from the melting transitions measured by differential scanning calorimetery (DSC) (T_m = 32 °C for polyester $\mathbf{14_d}$ compared to T_m = 30 °C for PEG-1100 monomethyl ether).

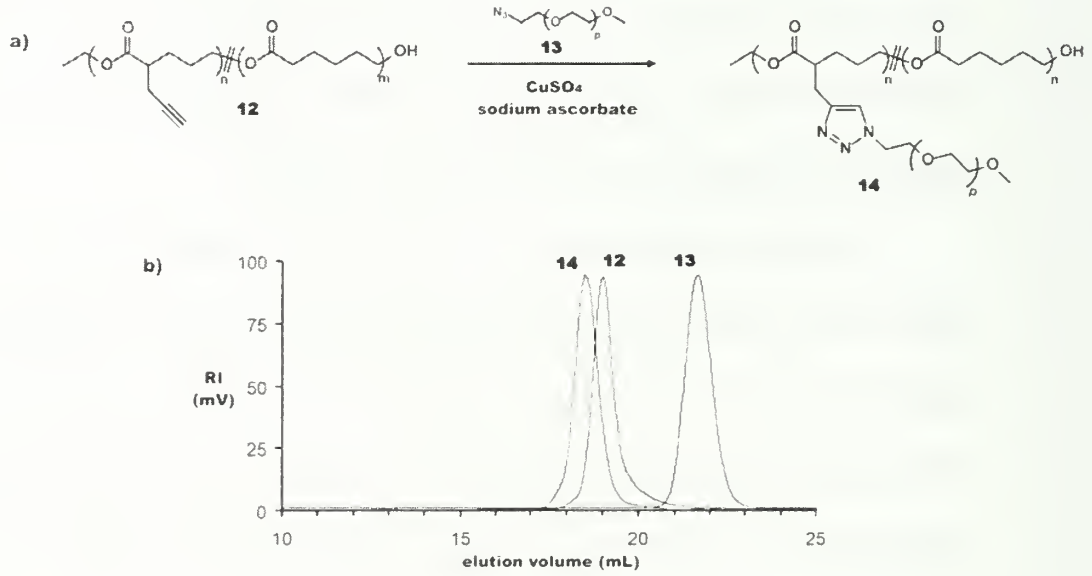


Figure 3.5 a) Synthesis of PEG-grated polyesters, b) overlaid GPC traces of acetylene-functionalized polyester 12, PEG-azide 13, and PEG-grafted polyester 14

Successful PEGylation via click cemistry was confirmed by the absence of azide and acetylene signals (2105 and 3280 cm^{-1} respectively) in the FTIR spectrum of the isolated product, and the appearance of a singlet for the methine proton of the triazole ring at 7.61 ppm in the ^1H NMR spectrum of **14**. Resonances at 144.4 and 123.5 ppm

were found in the ^{13}C NMR spectrum of **14**, corresponding to the carbons of the triazole ring. The fact that only two resonances are observed for these carbons confirms the formation of only one triazole regioisomer. The overlaid GPC traces of acetylene-functionalized polyester $\mathbf{12_d}$, PEG-azide **13**, and PEG-grafted polyester $\mathbf{14_d}$ are shown in Figure 3.5b. The narrow molecular weight distribution of $\mathbf{14_d}$ is particularly notable, and indicative of the tolerance of aliphatic polyesters towards click conditions.

The series of PEG-grafted polyesters $\mathbf{14_{a-d}}$ shown in Table 3.2, prepared from acetylene-functionalized precursors $\mathbf{12_{a-d}}$, all displayed an increase in molecular weight and maintained narrow polydispersities. The importance of this finding cannot be overstated, as there are very few examples of coupling chemistries that can be carried out cleanly on aliphatic polyesters.

Table 3.2 Characterization of PEG-grafted polyesters 14

Polymer	M_n x 10^3 g/mol[a]	PDI[a]	PEG grafts/polymer chain[b]	Grafting Efficiency[c]
$\mathbf{14_a}$	10.9	1.14	5	74%
$\mathbf{14_b}$	15.1	1.11	12	86%
$\mathbf{14_c}$	16.3	1.13	24	85%
$\mathbf{14_d}$	15.2	1.13	43	98%

[a] determined by GPC relative to polystyrene standards; [b] determined from ^1H NMR spectra; [c] estimated from GPC and ^{1}NMR data of acetylene functionalized starting materials

The PEG graft densities for copolymers $\mathbf{14_{a-d}}$ were calculated from their ^1H NMR spectra by integration of the methyl resonance at 3.35 ppm (PEG OCH_3), against the methylene resonance at 4.03 ppm ($CH_2CH_2OC(O)$ polyester backbone). The product of the PEG-to-polyester backbone ratio, and the degree of polymerization, gives the average number of PEG grafts per polyester chain. It is clear that a high degree of

PEG grafting density can be achieved and furthermore can be tailored by the amount of pendent acetylene in the polymer starting material. Grafting efficiencies were estimated by comparing the calculated PEG-grafting densities to the average number of acetylene groups per polyester backbone available for grafting in the starting material (given by the product of the molar incorporation of acetylene functionality and the degree of polymerization). The grafting efficiencies were found to be quite high (74-98%).

It is interesting to note the change in GPC-derived molecular weights of the PEG-grafted polyesters as a function of PEG-grafting density. In polymer $\mathbf{14_a}$, with low graft density, the observed molecular weight (10.9×10^3 g/mol) is close to the expected molecular weight calculated for the addition of 5 PEG 1100 chains to polymer $\mathbf{12_a}$ (13.4×10^3 g/mol). However, in polymer $\mathbf{14_d}$, with high graft density, the GPC-derived molecular weight (15.2×10^3 g/mol) is much less than the molecular weight calculated for the addition of 43 PEG 1100 chains to polymer $\mathbf{12_d}$ (53.3×10^3 g/mol). This underestimation in molecular weight must be due to the impact of grafting density on hydrodynamic radius, which gives these graft copolymers more compact structures than the linear standards used to calibrate the GPC. This finding is not surprising given the known influence of branching on GPC-deterived molecular weight in the case of dendrimers and hyperbranched polymers.[14]

Sequential PEG-grafting of homopolymer $\mathbf{12_d}$ was also performed. In the first coupling, 50% grafting was targeted employing the same click conditions described previously, but using 50 mole percent PEG-azide **13** relative to the acetylene-functionality of polymer $\mathbf{12_d}$. Following purification by dialysis, to remove a very small amount of unreacted **13**, and precipitation, a PEG-grafting density of 48% was

calculated from the ^{1}H NMR spectrum of the isolated product, by the integration of the methyl resonance at 3.35 ppm (PEG OCH_3), against the methylene resonance at 4.03 ppm ($CH_2CH_2OC(O)$ polyester backbone). In this copolymer, a resonance at 2.01 ppm corresponding to the acetylene functionality (C≡C-H) was observed and integration indicated that approximately 50% of the acetylene groups remained intact. Following this first grafting reaction, a second grafting, targeting the remaining acetylenes, was performed. After final purification the total PEG-grafting density was calculated at 80%. ^{1}H NMR and FTIR spectroscopy indicated no residual acetylene was present, by the absence of the characteristic signals at 2.01 ppm and 3280 cm^{-1} respectively. Only two resonances at 144.4 and 123.4 ppm were observed in the ^{13}C NMR spectrum of this sequentially PEGylated polyester indicating the formation of only one triazole regioisomer. While the grafting efficiency of this sequential method was not as high as that observed for complete single step grafting of homopolymer $\mathbf{12_d}$, it importantly demonstrates that the acetylene groups can be partially reacted, leaving the remaining acetylenes intact for further coupling.

3.3.2 Solution properties of PEG-grafted polyesters of type 14

The amphiphilic nature of PEG-grafted polyesters of type **14** distinguishes them from both the starting materials and conventional aliphatic polyesters. These copolymers are soluble in alcohols, water, and aqueous buffers, as well as most common organic solvents e.g. acetone, THF, CH_2Cl_2, and DMF. The solution structure of the graft copolymers was probed by ^{1}H NMR spectroscopy in $CDCl_3$ and D_2O as shown for copolymer **14b** in Figure 3.6. In $CDCl_3$, the copolymer is thouroughly

solvated, resulting in the clear splitting of the polyester backbone signals at 4.03 and 2.29 ppm, and a sharp singlet at 3.40 ppm for the terminal PEG methyl (Figure 3.6a). In contrast, ^{1}H NMR spectroscopy of PEG-grafted polyester **14b** in D_2O reveals broadened signals for the polyester component, indicating a poorly solvated, collapsed structure for the relatively hydrophobic polyester. The singlet at 3.40 ppm for the hydrophilic PEG methyl remains sharp in D_2O that this portion is well solvated in both solvents. In addition, the methylene group adjacent to the triazole ring is found at ~4.50 ppm in both spectra.

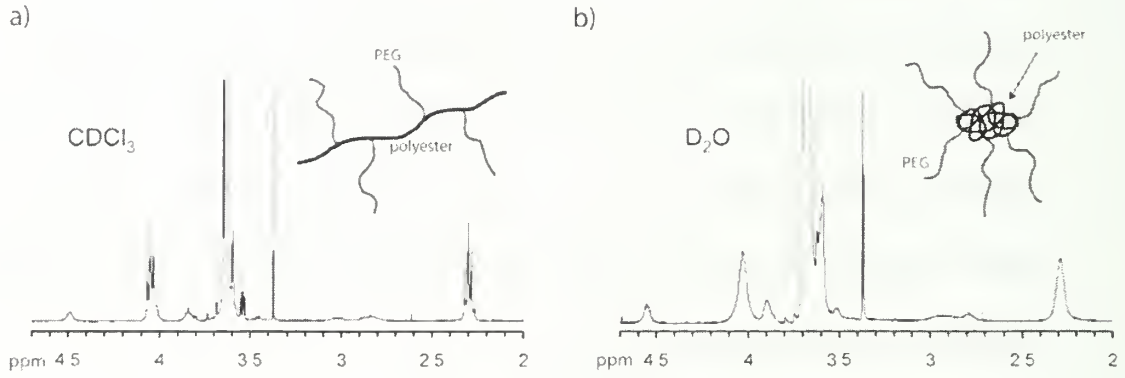


Figure 3.6 ^{1}H NMR spectra of PEG-grafted polyester 14_b in a) $CDCl_3$, and b) D_2O

To investigate the size of these collapsed PEG-grafted polyester structures dynamic light scattering was performed on aqueous solutions of polyester $\mathbf{14_b}$ to determine the hydrodynamic radius (R_h). Solutions of 5.0, 2.5, 1.25, and 0.25 mg/ml were prepared using water purified by a Mill-Q UF system, and filtered to remove dust particles. The normalized scattering intensity correlation function was then measured

as a function of angle from 55-135° in 10° increments. The decay time distribution function and the characteristic decay time (τ_0) at each angle were calculated using a nonlinear exponential fitting algorithm. Data was collected for each concentration at each angle three times and the average τ_0 was recorded. The relationship between τ_0 and the diffusion coefficient (D) is given by Equation 3.1.[15]

$$\frac{1}{\tau_o} = D * q^2$$

Equation 3.1

Thus, a plot of τ_0^{-1} versus the square of the scattering vector q, which is related to the scattering angle by $q=4\pi\sin(\theta/2)/\lambda$, affords the diffusion coefficient as the slope. The diffusion coefficient is related to R_h by Equation 3.2, the Stokes-Einstein equation.[15]

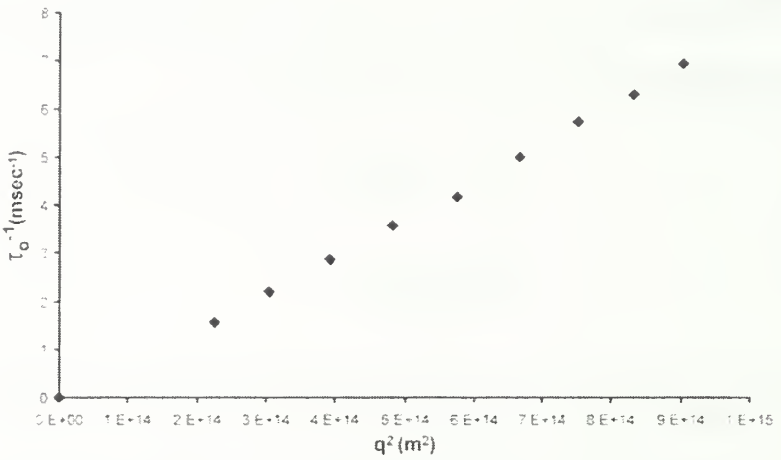
$$R_h = \frac{kT}{6\pi\eta D}$$

Equation 3.2

The plot of τ_0^{-1} as a function of q^2 is shown in Figure 3.7a for polymer **14**$_b$ at 1.25 mg/ml concentration in water. The plot is linear through the entire range of angles (55-135°), and the slope of the plot at different concentrations is very consistent to afford R_h values in the range of 32.3-33.4 nm, as shown in Figure 3.7b. The hydrodynamic radius is crucially important when considering drug delivery strategies and the value obtained of ~33 nm is on the order of polymer micelles and drug conjugates currently being investigated for endocytotic drug delivery systems. Additionally, the synthetic preparation of these PEG-grafted polyesters allows for adjusting the length of the

polyester backbone (n = 20-120), the length of the PEG-grafts (n = 3-45), and the density of PEG-grafting, which should provide a means for tuning R_h.

a)



b)

[polyester 12d]	R_h
0.25 mg/ml	33.0 nm
1.25 mg/ml	32.3 nm
2.5 mg/ml	33.4 nm
5.0 mg/ml	33.2 nm

Figure 3.7 a) Plot of τ_0^{-1} *vs.* q^2 for 1.25 mg/ml polyester 12_d obtained from dynamic light scattering data, b) R_h at different concentrations calculated from the Stokes-Einstein equation

3.3.3 Biocompatibility of PEG-grafted polyesters of type 14

An evaluation of these new synthetic polymers with regards to biocompatibility is critically important for judging their potential use in medical applications. This required selecting a relatively simple means to determine biocompatibility, and setting up the facilities to do so in the Emrick labs. These methods are intended as a first-stage evaluation of biocompatibility to indicate whether these new materials should be considered further for biological applications.

Cytotoxicity testing using mammalian fibroblasts is a commonly used *in-vitro* method for evaluating biocompatibility. Such testing requires methods and facilities to culture mammalian cell lines, and a means to evaluate cytotoxicity both qualitatively

and quantitatively. The equipment required for cell culture and handling, including an incubator, laminar flow hood, centrifuge, and autoclave were purchased and assembled in our labs. Standard aseptic techniques based on known protocols were employed in cell-culture.[16] Cytotoxicity was evaluated qualitatively by imaging cells with an inverted microscope equipped with a camera and phase-contrast accessories. Quantitative cytotoxicity evaluation was made by measuring hemolytic activity towards human red blood cells by UV-vis spectroscopy.

3.3.3.1 Minimal essential medium testing

The minimal essential medium (MEM) test is a qualitative measure of cytotoxicity. The test consists of growing a monolayer of fibroblasts to confluence in MEM, exposing the cells to the material in question, then observing the cells microscopically for changes in confluence and morphology. Specifically, mouse fibroblast cells (L929 purchased from American Type Culture Collection) were grown to >70% confluence in modified Eagle's medium supplemented with 10% horse serum (pH 7.0). The medium was removed and the water soluble PEG-grafted polyesters were introduced with fresh medium to give a final concentration of 5 mg/mL. After incubation for 24 hours, the cell monolayers were observed microscopically, and compared to positive and negative controls that used medium alone, and 5 mg/mL sodium dodecylsulfate (SDS), respectively. Full protocols for cell-culture and MEM testing are found in the Chapter 5. Phase-contrast optical micrographs taken after 24 hours exposure to polymers $\mathbf{14}_{a\text{-}d}$, along with positive and negative controls, are shown in Figure 3.8. Cells exposed to a cytotoxic material disengage from the cell culture

surface, develop a rounded morphology, and do not multiply. Low cell density, large numbers of rounded cells, and in the most extreme case burst cells were observed for the negative control in Figure 3.8, indicative of a cytotoxic response.

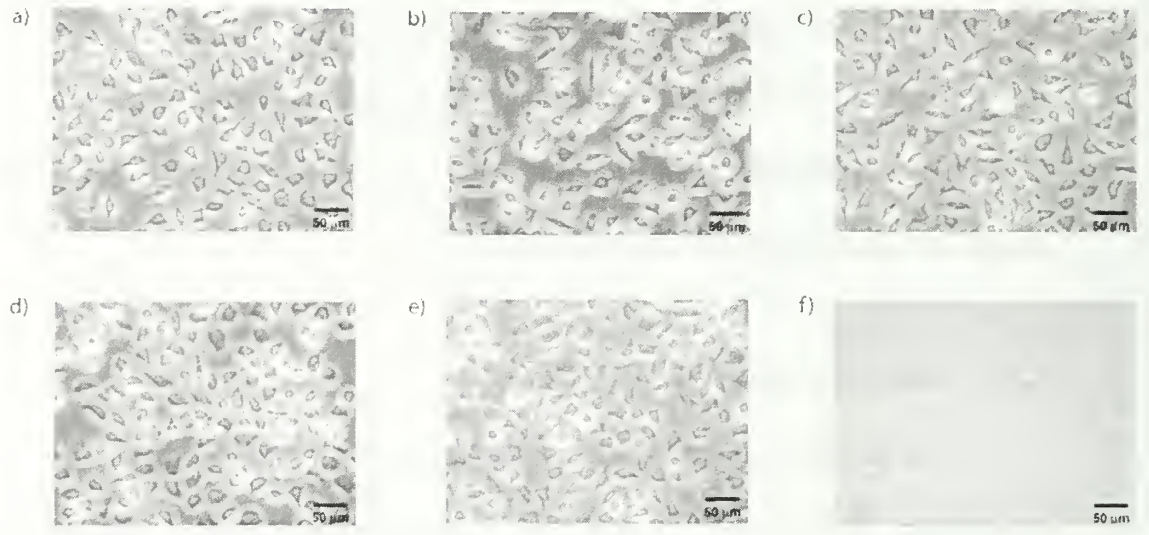


Figure 3.8 Minimal essential medium testing, 5 mg/mL polymer a) 14_d, b) 14_c, c) 14_b, d) 14_a, e) medium (positive control), f) 5 mg/mL sodium dodecylsulfate (negative control)

In contrast, the micrographs of cell culture experiments using polymers $\mathbf{14_{a-d}}$ show healthy cells that spread on the polystyrene tissue culture surface, at similar cell density and morphology to the positive control, indicating no cytotoxic response. Thus, qualitatively PEG-grafted polyesters of type **14** are not cytotoxic and are suitable for further investigation as biomaterials.

3.3.3.2 Hemolysis of human red blood cells

To determine the hemolytic activity of a material on red blood cells, the cells must first be separated from plasma by repeated centrifugation and resuspension in PBS. The red blood cells are then incubated with the material in question, centrifuged

to remove intact cells, and the absorbance of the supernatant measured spectroscopically. Cell lysis caused by cytotoxic material leads to release of heme into solution, which is detected by its absorbance at 413 nm, and compared to control experiments performed in the absence of the synthetic material to give a quantitative scale. Red blood cells incubated with pure water (which lyses the cells due to osmotic pressure) gives a value for complete lysis; other values are given as a relative percentage of complete lysis based on relative intensity of the heme absorbance. Cells that are incubated in PBS alone give a background absorbance, virtually no lysis, which is subtracted from all other values. The hemolytic activity of PEG-grafted polyesters $\mathbf{14}_{a\text{-}d}$ are shown in Figure 3.9, along with the results obtained for PEG-monomethyl ether-1100 and SDS as positive and negative controls respectively.

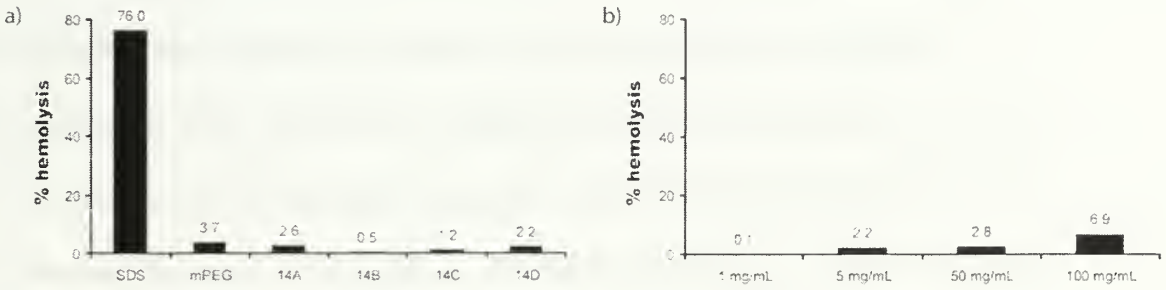


Figure 3.9 Hemolysis of PEG-grafted polyesters, a) 5 mg/mL with sodium dodecyl sulfate (SDS) and PEG monomethyl ether 1100 (mPEG) shown as negative and positive controls, b) hemolysis of polymer 14d as function of concentration

Less than 3% lysis was observed for PEG-grafted polyesters $\mathbf{14}_{a\text{-}d}$ at a concentration of 5 mg/mL, with the lowest value (0.5%) measured for polymer $\mathbf{14}_{b}$. All the measured hemolysis values are on the order of the 3.7% lysis measured for PEG-monomethyl ether-1100, which is generally accepted as biocompatible, and

dramatically less than the 76% lysis obtained for the negative control (Figure 3.9a). The effect of polymer concentration on hemolytic activity was determined for polymer $\mathbf{14}_{d}$ (Figure 3.9b). At low concentration (1 mg/mL), hemolysis was almost undetected (0.1%). Increasing the polymer concentration by two orders of magnitude (100 mg/mL) gave a modest hemolysis of 6.7%. These results provide a quantitative indication that PEG-grafted aliphatic polyesters of type **14** are not cytotoxic. Thus, both MEM and hemolysis data are encouraging with respect to the biocompatibility of these graft copolymer structures, and suggest further consideration of these materials for biomaterial applications. However, further *in-vivo* testing, as well as testing of all degradation products will be necessary.

3.4 Oligopeptide grafting of acetylene-functionalized polyesters of type 12 by click chemistry

Grafting of oligopeptide sequences to aliphatic polyesters would enable a "bio-tailoring" of these materials, which could enhance their use in many applications, such as tissue engineering, implanted materials, and targeted drug delivery. However, the grafting of aliphatic polyesters with deprotected oligopeptide sequences presents a particularly delicate challenge. Coupling of protected oligopeptide sequences to aliphatic polyesters followed by deprotection would result in degradation of the polyester backbone. Conversely, the multiple functionalities present in oligopeptide sequences result in polymer cross-linking when coupling deprotected oligopeptides. There is only one prior example of oligopeptide grafting to aliphatic polyesters, reported by Langer and coworkers, by copolymerization of L,L-lactide with an L-methyl-2,5-morpholinedione bearing a protected lysine residue.[17] Deprotection of the

lysine residue was incomplete and resulted in partial degradation of the polyester backbone. While a small amount of grafting of the deprotected oligopeptide sequence Gly-Arg-Gly-Asp-Tyr was achieved by diisopropylcarbodiimide coupling, cross-linking was also observed, presumably due to coupling at both the amine of the lysine residue and the amine terminus of the oligopeptide sequence. Click chemistry appeared to be a possible solution to this challenge due to its selectivity and mild reaction conditions.

In order to utilize click chemistry for oligopeptide grafting, an azide-terminated, deprotected oligopeptide sequence was synthesized by Rebecca Breitenkamp in the Emrick group. The sequence Gly-Arg-Gly-Asp-Ser (GRGDS) with a terminal azide (**15**) was prepared by standard solid phase peptide synthesis starting from a serine-loaded Wang resin and utilizing the peptide coupling agent HBTU, as depicted in Figure 3.10.[18] The GRGDS peptide sequence was chosen for its well-known ability to promote cell adhesion, but the method is not specific to this sequence.[19] The amine terminus of the resulting GRGDS oligopeptide sequence was capped with 6-bromohexanoic acid, and cleaved from the resin with 88/2/5/5 trifluoroacetic acid/triisopropylsilane/ H_2O/phenol, to give the bromide-terminated oligopeptide sequence. Reaction with sodium azide in DMSO afforded azide-terminated Gly-Arg-Gly-Asp-Ser **15** in 98% yield (based on the resin loading density). The structure of **15** was confirmed by high resolution mass spectrometry (HRMS-FAB (m/z): $[M+H]^+$ calculated 630.296, found 630.296), as well as NMR and FTIR spectroscopy.

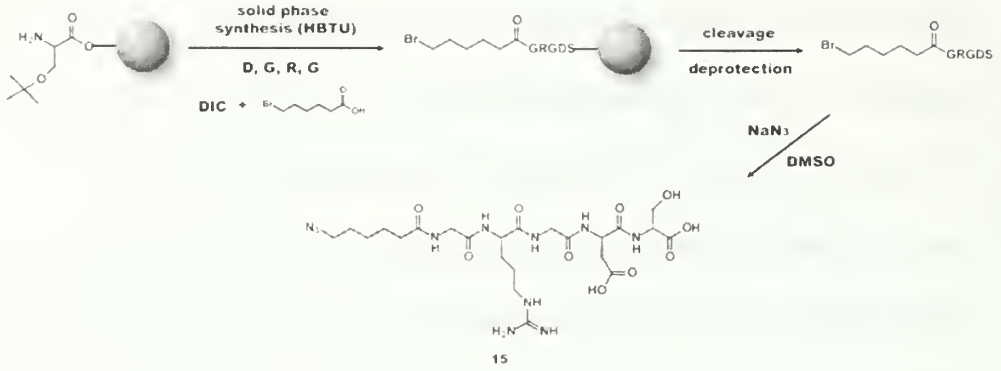


Figure 3.10 Synthesis of azide end-capped GRGDS-oligopeptide

Initial attempts to synthesize oligopeptide-grafted aliphatic polyesters focused on the acetylene-functionalized homopolymer $\mathbf{12_d}$ to give copolymer $\mathbf{16_d}$, where 5% peptide grafting was targeted (Figure 3.11). The procedure employed for this click reaction was similar to that described for PEG-grafting, except that higher temperatures (ca. 100 °C) were generally needed for successful triazole formation in the oligopeptide case. Successful oligopeptide grafting was confirmed from the ^{1}H NMR spectrum of polymer $\mathbf{16_d}$ taken in $CDCl_3$, by the presence of signals in the range of 6.70-7.80 ppm, corresponding to the peptide amide protons, and the triazole proton. The appearance of these signals in $CDCl_3$ is particularly telling as the free oligopeptide **15** is insoluble in chloroform and ^{1}H NMR spectra of mixtures of acetylene-functionalized polymer $\mathbf{12_d}$ and azide-functionalized oligopeptide **15**, recorded in $CDCl_3$, showed no sign of these resonances. To further support peptide grafting, and investigate the integrity of the functionalized aliphatic polyester over time, GPC analysis was performed in DMF as shown in Figure 3.11.

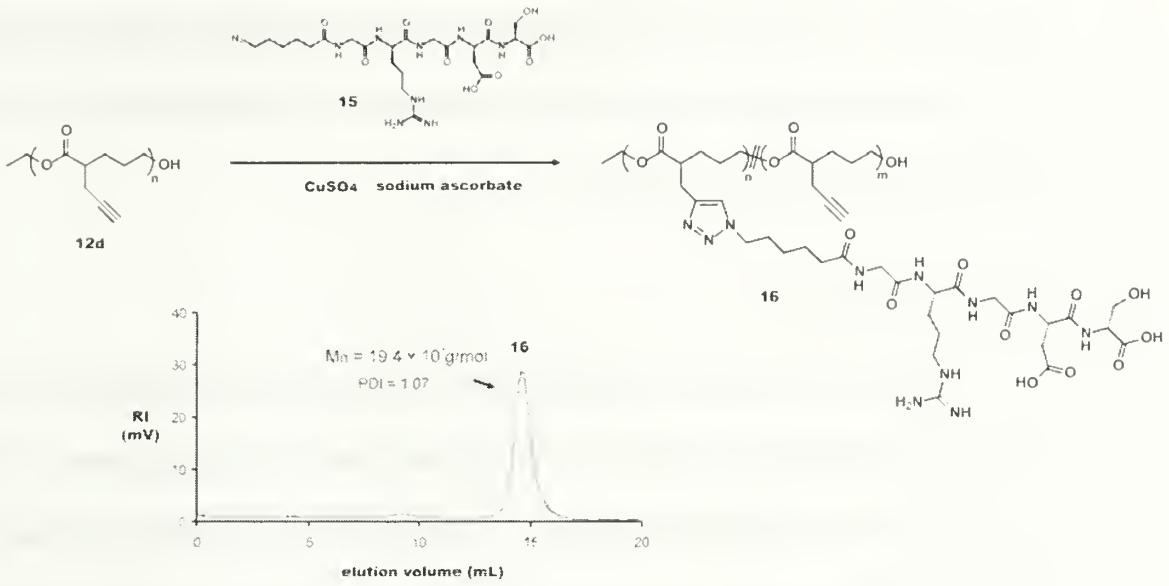


Figure 3.11 Top: oligopeptide grafting of polyester 12_d; bottom: GPC chromatogram in DMF of isolated product after storage for 30 days

No substantial change in polydispersity occurred following the click grafting reaction indicating the absence of degradation or cross-linking. Moreover, it appears that these polyesters have good shelf stability (starting material $\mathbf{12_d}$: M_n = 20.4 x 10^3 g/mol, PDI = 1.07; GRGDS-grafted polymer $\mathbf{16_d}$ after 1 month storage at ambient conditions: M_n = 19.4 x 10^3 g/mol, PDI = 1.07). The weak signal at low retention time (high molecular weight) in the GPC trace is attributed to aggregation of the oligopeptide-functionalized aliphatic polyester, as the GPC was run in DMF with no added salts.

The synthesis of aliphatic polyesters with a higher degree of oligopeptide grafting was attempted using 20 mole percent oligopeptide **15** relative to the acetylene-functionality of homopolymer $\mathbf{12_d}$. Additionally, oligopeptide grafting to a PEG-grafted polyester of type **14** (40 mole percent PEG-grafting and 60 mole percent residual acetylene functionality) was attempted. Both experiments gave insoluble

polymer films. This may be due to hydrogen bonding among the multiple oligopeptide grafts per polymer chain, but this is only speculation as no spectroscopic confirmation of oligopeptide grafting was obtained.

3.5 Future outlook

While the versatility of click chemistry in the grafting of aliphatic polyesters has been demonstrated in this work, the variety of functional groups incorporated by this method could be expanded considerably. The ability to react a fraction of the acetylene groups of a given polyester sample, while leaving the remaining acetylenes intact for subsequent reaction, could also be utilized to advantage in many applications. For example, this would allow for grafting of drug moieties, PEG-chains, and targeting oligopeptide sequences to the same polyester backbone, which would then function as a degradable drug-delivery vehicle with enhanced circulation time and increased targeting efficiency of tumor cells. Experiments along these and related lines are the topic of Chapter 4.

3.7 References

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CHAPTER 4

FUNCTIONALIZED ALIPHATIC POLYESTERS FOR INTERFACIAL ASSEMBLY, ENCAPSULATION, AND POLYMER THERAPEUTICS

The methods for polyester functionalization presented in previous chapters provide synthetic control and diversity that can be applied to polymer-drug conjugates and microparticle delivery systems. A common example of a polymer-drug conjugate is the covalent binding of a single drug molecule to a PEG chain through an ester linkage. The PEG chain enhances solubility and *in-vivo* circulation time, and reduces phagocytic clearance, while the ester linkage enables release of the drug upon degradation.[1] PEG-grafted polyesters that incorporate drug grafts, should also exhibit enhanced solubility and circulation time, but in addition provide the opportunity to graft a large number of drug molecules to a single polymer backbone. Moreover, the distribution of PEG chains along the polyester backbone, rather than only at the chain end, should lead to the release of smaller polyester/PEG-drug conjugates upon degradation. This could potentially afford a longer and better controlled release. In addition, the narrow polydispersities, of these PEG-grafted polyester structures, should provide a greater degree of pharmacokinetic reproducibility when compared to more polydisperse systems.

Polyester-PEG micelles and polyester microparticles used as reservoirs for hydrophobic drugs, release drug based on diffusion, degradation, and stability in the case of micelles. Consequently such systems often suffer from a burst release based on micelle destabilization or rapid drug diffusion from microparticles.[2-3] Comparatively, micelles or microparticles prepared from PEG-grafted polyester conjugates with

covalently bound drug molecules should not exhibit burst effects as upon degradation or destabilization smaller drug-conjugates will be released, rather than the small molecule drug itself. Furthermore, for covalently bound drugs, release should be dominated by degradation rather than diffusion, which may impart a greater degree of control. Residual acetylene functionality in these conjugates can be used for cross-linking, which should provide even greater stability and slower release. This chapter centers on the preparation of polyester-drug conjugates, and cross-linked polyester microparticles from PEG-grafted polyesters containing residual acetylene functionality.

4.1 Aliphatic polyester-drug conjugates prepared by click chemistry

The covalent binding of small molecule drugs to polymers is limited by the functionality of the drug moiety, and the compatibility of the coupling chemistry with both the drug and polymer structure. The versatility and mild conditions of click chemistry offer enormous potential for conjugating drugs to acetylene-functionalized polyesters. This chapter focuses on polyester functionalization with 20-(S)-camptothecin, an anti-cancer drug extracted from the Asian tree *Camptothica acuminate* (Figure 4.1). Camptothecin was first isolated by Wall and coworkers in 1966 and displayed remarkable cytotoxic activity against a range of cancers in animal models.[4-5] However, in human clinical studies the drug performed poorly, yielding little therapeutic effect, and many negative side effects. Due to the poor water/plasma solubility of camptothecin, the sodium salt of the open acid form was used in human trials.

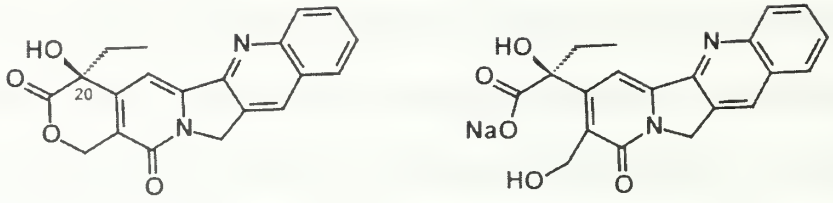


Figure 4.1 Structures of 20-(S)-camptothecin (left) and the sodium salt of the ring-opened form (right)

However, subsequent studies determined that the closed lactone ring and the hydroxyl group at the 20 position are vital to anti-tumor activity.[6-7] The poor solubility of camptothecin is further complicated by the fact that in human plasma at pH ~ 7.4 it is rapidly converted to the open acid form and thus not active. Efforts to improve the solubility of camptothecin resulted in Irinotecan and Topotecan, water soluble 10-OH derivatives that were approved for human use in 1997.[8-9]

Greenwald and coworkers recently reported that esterification at the 20-OH position improves both the solubility and lactone stability of camptothecin.[10] PEG-esterification was shown to stabilize camptothecin in the lactone form, until exocylic ester cleavage occurred at which point the drug was released and rapidly converted to the open acid form as depicted in Figure 4.2.

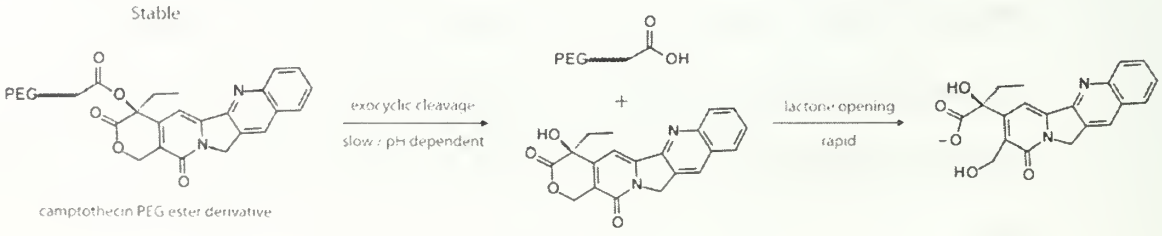


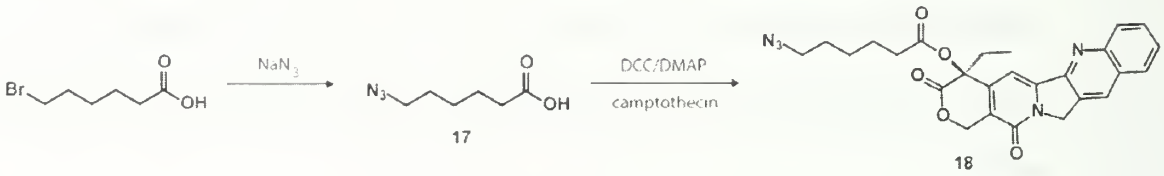
Figure 4.2 Camptothecin esterified at the 20-OH position with PEG and release upon exocyclic cleavage

Greenwald's report suggests that a well-defined polymer-based camptothecin delivery system, with a 20-OH ester linkage, will improve drug solubility and circulation time, and also provide a means of pH dependent drug release. The polyester-camptothecin conjugate would remain stable during circulation in plasma at pH ~ 7.4, and accumulate in cancerous tissue by means of the EPR effect. Upon cellular uptake the conjugate would rapidly degrade in the lysosome at pH ~ 4-5 and release the active form of the drug. The development of an appropriate design is thus the focus of the following section.

4.1.1 Synthesis of camptothecin-azide derivative

6-Bromohexanoic acid was chosen to link camptothecin to polyester backbones based on the successful azide functionalization of oligopeptide sequences and subsequent click chemistry reported in Section 3.4. In contrast to the oligopeptide synthesis, where the carboxylic acid linker was coupled first and the oligopeptide-azide introduced at the last step, for camptothecin it was advantageous to prepare the 6-azidohexanoic acid first and subsequently couple to camptothecin (Scheme 4.1). This approach limits the synthetic chemistry performed in the presence of highly-toxic, free camptothecin. Nucleophilic substitution of 6-bromohexanoic acid with sodium azide was achieved in DMSO at room temperature, and the α,ω-azidoacid product purified by vacuum distillation to afford 6-azidohexanoic acid (**17**) as a colorless liquid. The transformation is clearly observed in the ^{1}H NMR spectrum, where the methylene triplet at 3.28 ppm (CH_2N_3) is shifted relative to the starting material methylene signal at 3.42 ppm (CH_2Br). The carboxylic acid is observed as a broad signal at 11.5 ppm in the ^{1}H

NMR spectrum and a signal at 180.13 ppm in the ^{13}C NMR spectrum. The signal at 51.19 ppm, in the ^{13}C NMR spectrum of **17**, is characteristic of aliphatic azides (CH_2N_3). The presence of the azide group was also confirmed in FTIR spectroscopy by the characteristic absorbance at 2108 cm^{-1}.

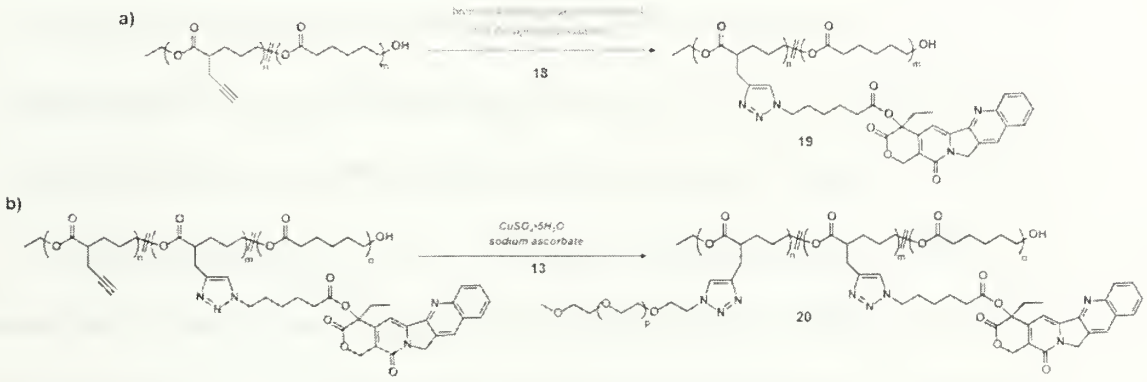


Scheme 4.1 Synthesis of 6-azidohexanoic acid 17 and subsequent coupling to afford camptothecin-azide derivative 18

Despite the limited solubility of camptothecin, DCC coupling was performed successfully in CH_2Cl_2 using 3 equivalents of DCC and 6-azidohexanoic acid **17**. The product was purified by precipitation into ether, followed by filtration and recrystallization from methanol to afford camptothecin-azide **18** in 87% yield as a slightly-yellow crystalline solid. The structure of **18** was confirmed by high resolution mass spectrometry (HRMS-EI (m/z): $[M]^+$ calculated for $C_{26}H_{25}N_5O_5$ 487.186, found 487.186.), as well as ^1H NMR, ^{13}C NMR, and FTIR spectroscopy. The signals corresponding to the methylene of the camptothecin lactone appear as doublets at 5.68 and 5.41 ppm in the ^1H NMR spectrum because they are not equivalent due to the other substituents of the ring. These signals are shifted slightly down-field relative to camptothecin and indicate that the lactone ring is intact.

4.1.2 Synthesis of polyester-camptothecin conjugates

Aliphatic polyester-camptothecin conjugates were synthesized by coupling camptothecin-azide **18** to acetylene-functionalized polyesters of type **12** to give hydrophobic conjugates of type **19**. Subsequent PEG-grafting of conjugates with residual acetylene functionality gave hydrophilic polyester-camptothecin conjugates of type **20** (Scheme 4.2).



Scheme 4.2 Synthesis of a) hydrophobic and b) hydrophilic polyester/PEG-camptothecin conjugates 19 and 20 respectively

Coupling of camptothecin-azide **18** to aliphatic polyesters of type **12** by click chemistry in water was unsuccessful, presumably due to the poor solubility of **18** in water. Recent reports by Hawker and Wooley have shown that click chemistry can be performed in organic solvents using copper catalysts with organic solubilizing ligands.[11-12] These reactions readily proceed to near full conversion, however they require longer reaction times (~48 h.). Using similar procedures, hydrophobic polyester-camptothecin conjugate $\mathbf{19_a}$ was prepared by coupling polymer $\mathbf{12_e}$ (10 mole percent acetylene, M_n 7.6 x 10^3 g/mol, PDI 1.18) to camptothecin-azide **18** in CH_2Cl_2 using *N,N*-diisopropylethylamine and bromotris(triphenylphosphine)copper(I). The reaction was

monitored by ^{1}H NMR and was complete after 48 h. The crude product was purified by precipitation into ether, dialysis in CH_2Cl_2, and then precipitation into cold hexanes to afford the polymer **19**$_a$ as an off white semi-crystalline solid. ^{1}H NMR spectroscopic analysis reveals signals at 8.39, 8.23, 7.93, 7.86, 7.64, 7.19, 5.65, 5.39, 5.29, and 0.99 ppm corresponding to camptothecin and 4.04 ppm corresponding to the polyester backbone. New signals are observed at 7.64 and 4.25 ppm corresponding to the triazole proton R_2C=C*H*R and the methylene adjacent to the triazole C*H*$_2$NR_2. No residual acetylene was observed as evidenced by the absence of a signal at 2.01 ppm. The relative integrations of the signals at 4.04 ppm (polyester) and 4.25 ppm (triazole) indicate 9 % camptothecin grafting was achieved. GPC analysis in THF shows a substantial increase in molecular weight (M_n = 11.9 x 10^3 g/mol) and a narrow polydispersity (PDI = 1.18) indicating that the coupling was achieved in the absence of degradation. Such hydrophobic polyester-camptothecin conjugates could be utilized to prepare degradable microparticles with covalently bound drug for enhanced control over release.

Hydrophilic PEG-grafted polyester-camptothecin conjugates were prepared in two steps. First camptothecin-azide **18** (4.1 x 10^{-1} mmol) was coupled to polymer **12**$_f$ (52 mole percent acetylene, M_n 9.2 x 10^3 g/mol, PDI 1.17, 500 mg, 2.1 mmol acetylene), with *N,N*-diisopropylethylamine and bromotris(triphenylphosphine)copper(I) and targeting 15 mole percent camptothecin grafting. Purification by precipitation and dialysis gave polymer **19**$_b$ (M_n = 14.3 x 10^3 g/mol, PDI = 1.19, 12 mole percent camptothecin grafting, 38 mole percent residual acetylene as determined from ^{1}H NMR spectroscopy by the relative integrations of

signals at 4.25 (CH_2NR_2 camptothecin **18**), 4.04 ($CH_2OC{=}O$ polyester backbone), 2.03 (-C≡C-H)). PEG-grafting of the residual acetylenes of polymer $\mathbf{19_b}$ was then performed, in water, as described previously for polymers of type **14** to give hydrophilic PEG-grafted polyester-camptothecin conjugate **20** as an slightly yellow powder. ^{1}H NMR spectroscopy of **20** indicated successful coupling of both PEG and camptothecin by the triazole proton signals at 7.66 and 7.51 ppm, corresponding to $R_2C{=}CH$ camptothecin and $R_2C{=}CH$ PEG respectively, and the signals for the methylene units adjacent to the triazole at 4.46 and 4.23 ppm corresponding to R_2NCH_2 PEG and R_2NCH_2 camptothecin respectively. The relative integrations of the signals at 4.46, 4.32, and 4.04 ppm correspond to 12 % camptothecin-grafting and 35% PEG-grafting. GPC analysis in THF gave a large increase in molecular weight (M_n = 23.5 x 10^3 g/mol) and narrow polydispersity (PDI = 1.20) indicating grafting in the absence of degradation. Polymer **20** was soluble in acetone, DMSO, CH_2Cl_2 as well as water. Presumably, these PEG-grafted polyester-camptothecin conjugates will exhibit similar solution behavior to that observed for PEG-grafted polyesters of type **14** described in Chapter 3. With R_h values on the order of 30 nm these conjugates should be suitable for targeting of tumor tissue by the EPR effect. Continuing efforts in the Emrick group will focus on the *in-vitro* degradation and release behaviors of these conjugates.

4.2 Cross-linked polyester microparticles prepared by click chemistry

Amphiphilic graft copolymers, such as the PEG-grafted aliphatic polyesters described in this thesis, lend themselves to many applications in the biomaterials/drug-delivery arena. The remainder of this chapter will describe efforts to use these

polymers, not only as drug carrying conjugates, but also as materials for encapsulation and controlled release. The ability of these polymers to assemble at and stabilize oil-water interfaces and the use of this behavior in the preparation of cross-linked microparticles will be discussed as depicted in Figure 4.3.

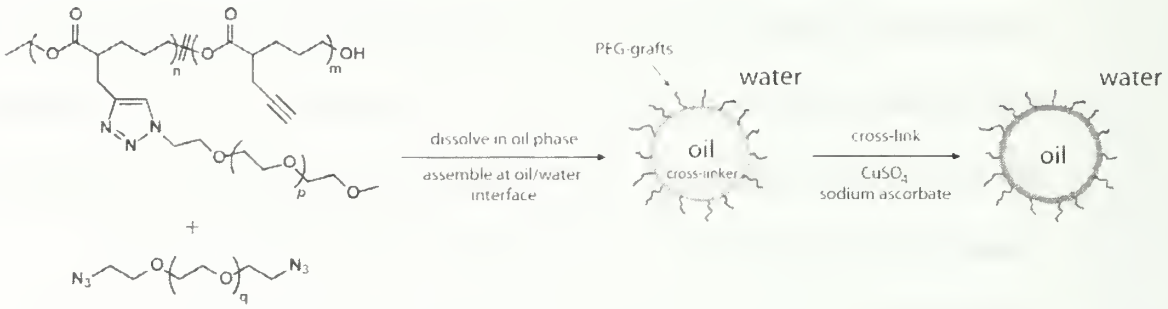


Figure 4.3 Self-assembly of PEG-grafted polyesters at oil/water interfaces and subsequent cross-linking to afford microcapsules

PEG-grafted polyesters of type **14** were shown to segregate to and stabilize oil/water interfaces by adding 100 μL of a 1% w/w solution of polymer **14c** in toluene to 20 mL of water in a glass vial and shaking vigorously. The resulting toluene droplets are stabilized by the PEG-grafted polyester and can be visualized by phase contrast optical microscopy as shown in Figure 4.4.

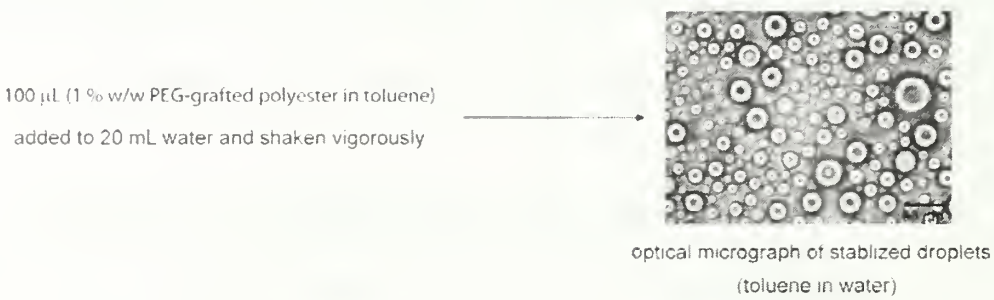
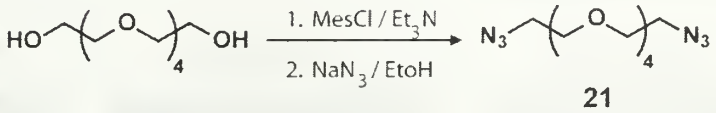


Figure 4.4 Assembly of PEG-grafted polyester at the toluene/water interface

In the absence of PEG-grafted polyester, the toluene droplets would quickly coalesce. Residual acetylene functionality in these materials should provide the means for cross-linking using click chemistry and a di-functional azide.

4.2.1 Preparation of cross-linked polyester microparticles

α,ω-PEG_{200}-diazide was chosen as the cross-linker so that this component would have some interfacial activity and segregate to the interface, but not be water-soluble such that inter-capsule cross-linking does not compete with interfacial cross-linking. PEG_{200}-diazide (**21**) was prepared by mesylation in THF with 2.5 equivalents of methanesulfonyl chloride, followed by nucleophilic substitution of the mesylates with sodium azide (3 equivalents) in refluxing ethanol, as depicted in Scheme 4.3.



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Scheme 4.3 Synthesis of α,ω-diazide-PEG_{200} as a cross-linker for interfacial assemblies of PEG-grafted polyesters with residual acetylene

Use of excess of methanesulfonyl chloride and sodium azide ensured high yields of the desired product, which could be isolated by extraction. Diazide **21** was obtained as a slightly yellow liquid, and the structure confirmed by ^{1}H NMR, ^{13}C NMR, and IR. In particular, the presence of the azide was confirmed by a characteristic signal at 2110 cm^{-1} in the FTIR spectrum, and a resonance at 51.1 ppm in the ^{13}C NMR spectrum, representative of CH_2N_3.

Polyester-stabilized oil-droplet assemblies in water were prepared by dissolving 0.25% w/w PEG-grafted polyester **14**$_e$ (25 mole percent PEG-grafting, 10% residual acetylene as calculated by ^{1}H NMR integration) in toluene and adding the diazide cross-linker **21** (0.5 mole percent relative to residual acetylene). Several drops (~100 μL) of the resulting toluene solution were than added to 20 mL of water in a small vial and shaken vigorously. The stabilized droplets were then imaged by phase contrast microscopy prior to cross-linking as shown in Figure 4.5. The size of the droplets assemblies was controlled by the method of shaking. Droplets that were shaken by hand, were typically 15-100 μm while droplets prepared by sonication were much smaller (5-25 μm) and uniform with respect to size. Droplets that were shaken by hand remained stable in water for long periods of time and did not show evidence of coalescence after 5 weeks.

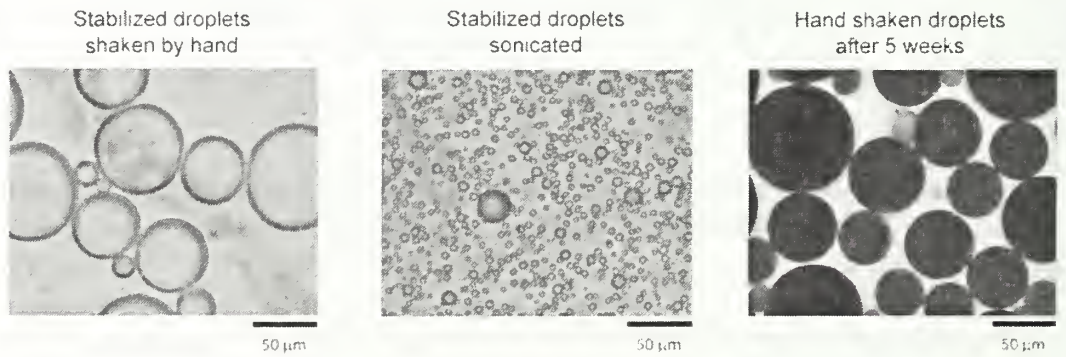


Figure 4.5 Phase-contrast micrographs of PEG-grafted polyester stabilized droplets (oil in water) containing diazide cross-linker

Cross-linking was performed by introduction of copper(II) sulfate and sodium ascorbate to the aqueous phase either at 20 or 40 mol % relative to the residual acetylene. The assemblies were gently agitated by swirling, to promote mixing of the reagents, and then allowed to stand for several hours. The cross-linked assemblies

were then washed by repeated removal of the aqueous phase and addition of fresh water. Phase contrast micrographs taken before and after cross-linking show little change in the assemblies due to the presence of the interface. To verify that the capsules were in fact cross-linked they were washed repeatedly with excess methanol to remove the interface. Without the interface present, uncross-linked assemblies are destroyed and the once stabilized droplets quickly coalesce. In contrast, the cross-linked assemblies retain some structural integrity upon removal of the interface, and are observed as spheroid particles or collapsed capsules depending on the thickness and strength of the capsule walls. Phase contrast micrographs before and after cross-linking, and after removal of the interface by addition of methanol, are shown in Figure 4.6a-c.

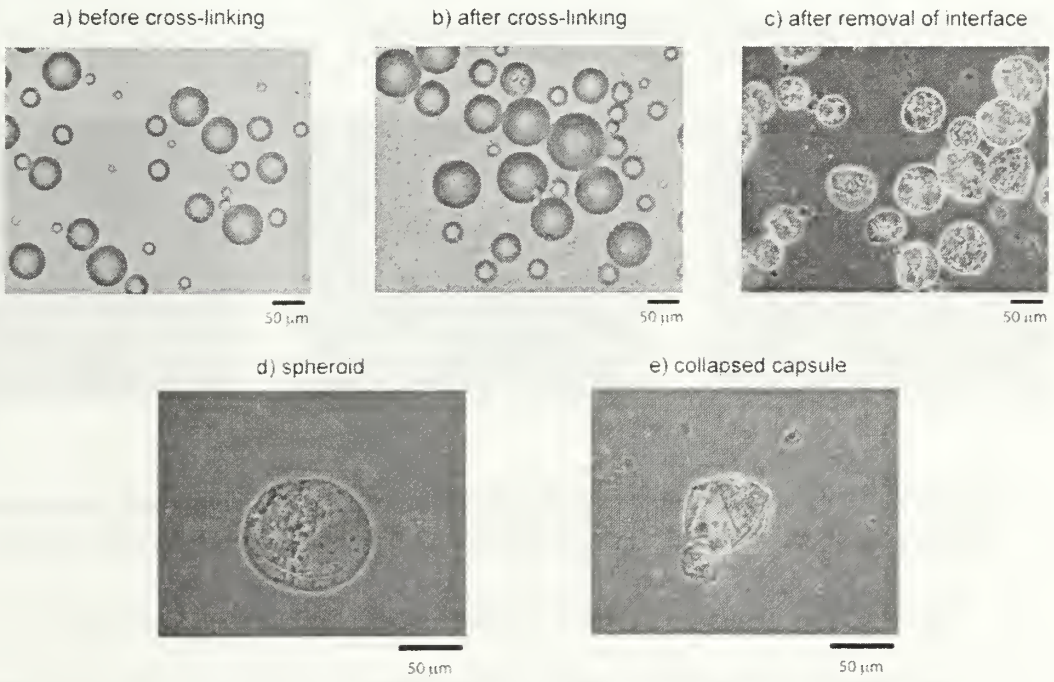


Figure 4.6 Phase contrast micrographs of stabilized droplet assemblies a) before cross-linking, b) after cross-linking, and c) after removal of the interface, d) and e) spheroid and collapsed capsule structures respectively

In Figure 4.6c some collapsed microcapsules are observed, but the majority are intact spheroids. The spheroids and collapsed microcapsule structures are more clearly observed at higher magnification, as shown in Figure 4.6d-e. The presence of intact spheroids indicates that either the cross-linked assemblies are thick walled microcapsules, or there is cross-linking occurring on the interior of the droplets and thus solid microparticles are formed. To probe this issue further capsules with a lower degree of cross-linking were prepared by adding 10 mole percent cross-linker relative to the residual acetylene functionality. Phase contrast micrographs of these capsules before and after cross-linking are almost identical to those observed for the higher cross-linking density. However, upon removal of the interface, at the lower degree of cross-linking the majority of the cross-linked assemblies appear collapsed, as shown in Figure 4.7, with only a few intact spheroids observed.

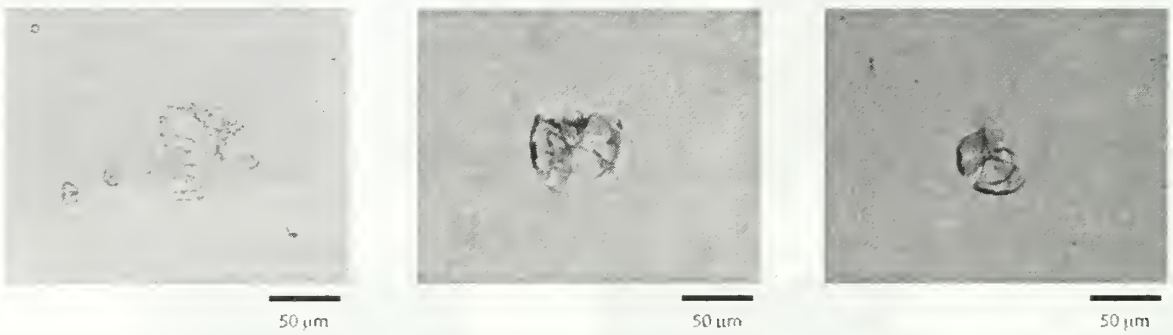


Figure 4.7 Phase contrast micrographs of collapsed microcapsule structures observed upon removal of the interface at a lower degree of cross-linking

These results suggest that cross-linking density can be used to control the mechanical integrity of the microcapsules upon removal of the interface which should have a dramatic effect on their degradation and drug release behavior. The stability of cross-linked polyester microparticles and the absence of inter-particle cross-linking was

further demonstrated by real time phase-contrast microscopy experiments. Samples of uncross-linked PEG-grafted polyester oil/water assemblies and cross-linked PEG-grafted polyester microparticles were prepared in concave microscope slides. On the microscope stage, excess methanol was added to each slide to remove the interface, while the process was digitally recorded. Upon removal of the interface, the uncross-linked PEG-grafted polyester assemblies quickly coalesce as the interface is destroyed, as shown in Figure 4.8.

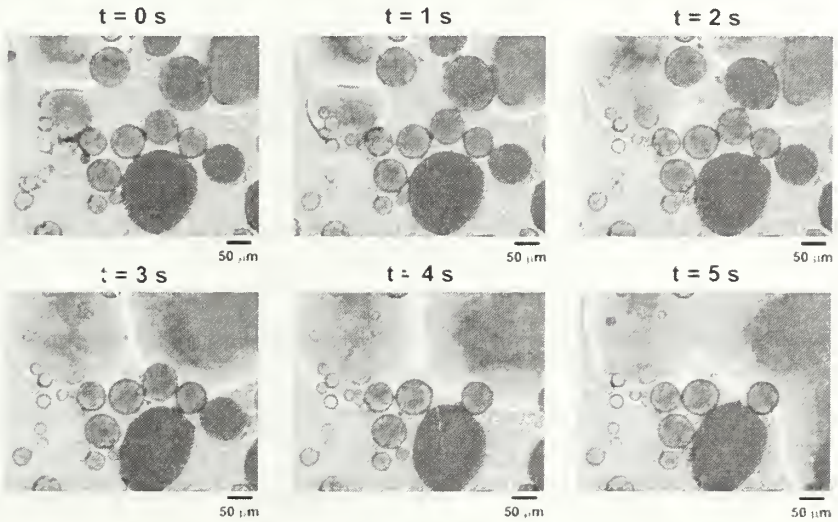


Figure 4.8 Phase-contrast microscope images showing coalescence of PEG-grafted polyester oil/water assemblies upon addition of excess methanol

In contrast, cross-linked PEG-grafted polyester microparticles do not coalesce, as shown in Figure 4.9. Motion, due to solvent heating by the microscope light, causes the microparticles to swirl around rapidly and collide violently with each other. These collisions demonstrate the stability of the cross-linked microparticles and the absence of inter-particle cross-linking.

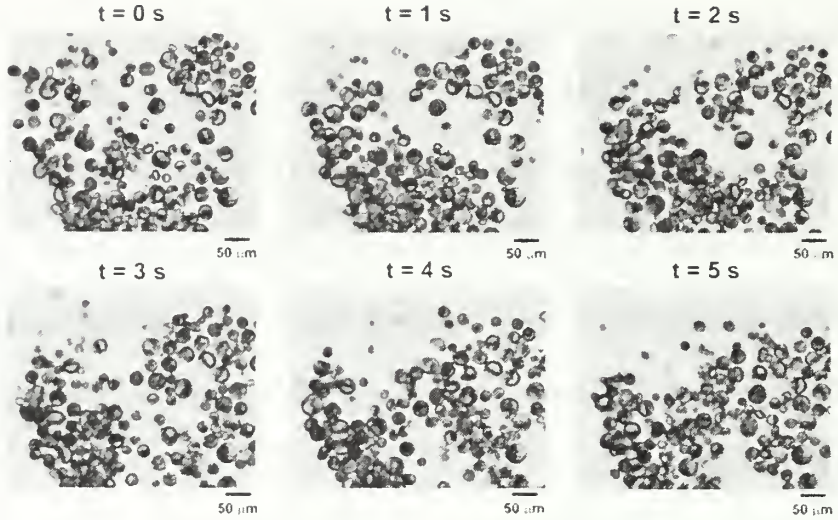
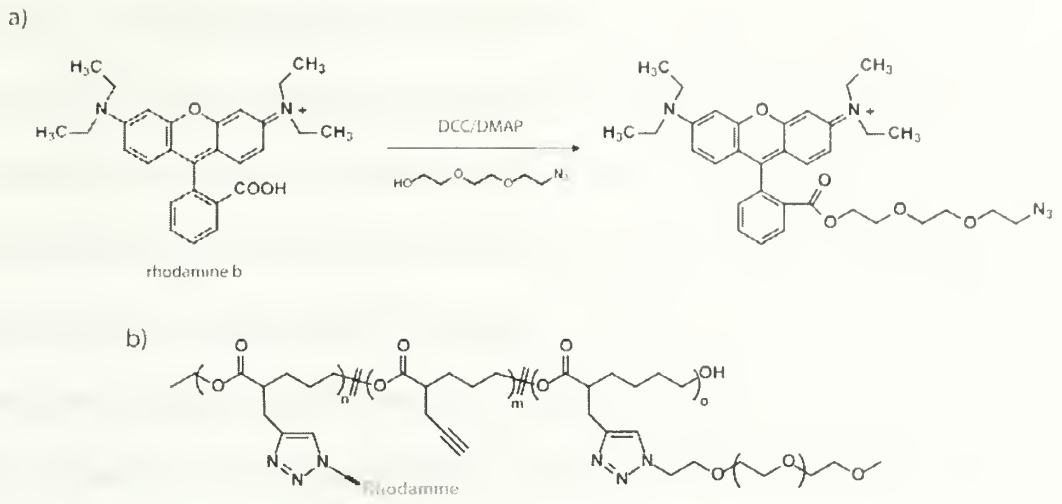


Figure 4.9 Phase-contrast microscope images showing cross-linked PEG-grafted polyester microparticles upon removal of the interface by the addition of excess methanol

4.2.2 Labeling of polyester microcapsules for confocal microscopy

In order to image the polyester microcapsules by confocal microscopy, an azide derivative of the fluorescent dye rhodamine-b (**22**) was synthesized and incorporated into PEG-grafted polyesters as shown in Scheme 4.4. The azide synthesis was complicated due to fluorescent impurities in the rhodamine-b starting material. As a result, the desired rhodamine azide derivate also contained these impurities. While pure **22** was never obtained, it was used solely for imaging purposes and the presence of impurities should not affect coupling to PEG-grafted polyesters. Coupling of the rhodamine-azide derivative was achieved under the same conditions used for PEG grafting described in Chapter 3. Purification by dialysis in water for 7 days removed most of the residual rhodamine-b impurities, and the dye-labeled PEG-grafted polyesters were assembled at oil/water interfaces and cross-linked as described previously.



Scheme 4.4 a) Synthesis of rhodamine-b azide, b) incorporation into PEG-grafted polyesters with residual acetylene functionality

Confocal microscope images of the assembled droplets before cross-linking and after cross-linking with the interface removed by washing with excess methanol are shown in Figure 4.10. When viewed under the confocal microscope, fluid filled, labeled microcapsules should appear as circular rings because each image is collected from an individual planar slice of the sample. The labeled polyester assemblies shown in Figure 4.10a however appear as filled circles possibly due to interior cross-linking.

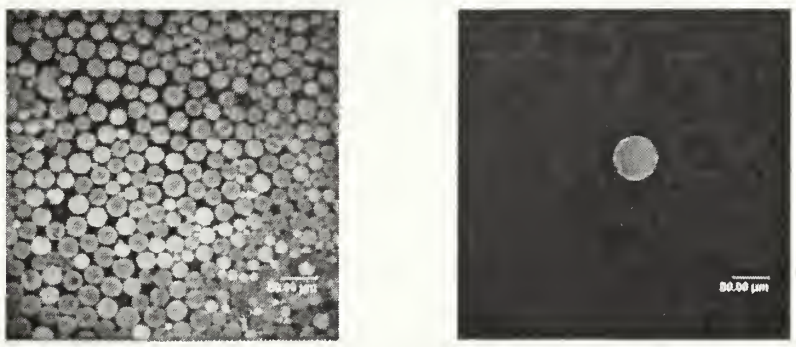


Figure 4.10 Confocal images of labeled PEG-grafted polyester a) droplet assemblies before cross-linking, b) microparticle after cross-linking and removal of the interface

Images collected at higher magnification and from several different slices of the microparticles reveal an uneven distribution of fluorescent material, as shown in Figure 4.11. The image collected from the bottom shows weak fluorescence throughout the microparticle, while the image collected from the middle of the microparticle shows strong fluorescence throughout. In contrast, the image collected from the top of the microparticle shows little fluorescence in the interior, with brighter fluorescence at the microparticle wall suggesting a hollow or fluid-filled structure. The uneven distribution of labeled polyester is likely an effect of the cross-linking conditions, which could be optimized to produce more uniform microparticles.

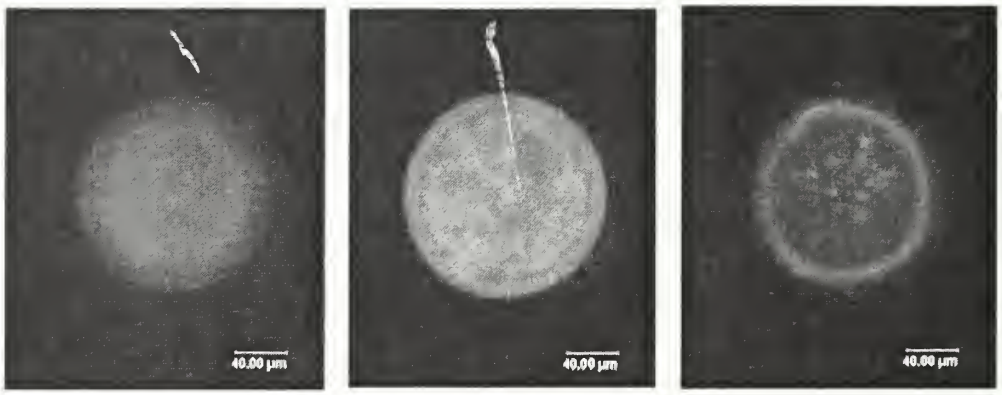


Figure 4.11 Confocal microscope images collected from the bottom (left), middle (middle), and top (right) of a cross-linked polyester microparticle

Encapsulation of camptothecin in PEG-grafted microparticles was demonstrated by preparing a solution of the drug in toluene with PEG-grafted polyester **14**$_e$ and diazide cross-linker **21**. Droplet assemblies were then prepared in water, cross-linked, and the interface subsequently removed as depicted in Figure 4.12.

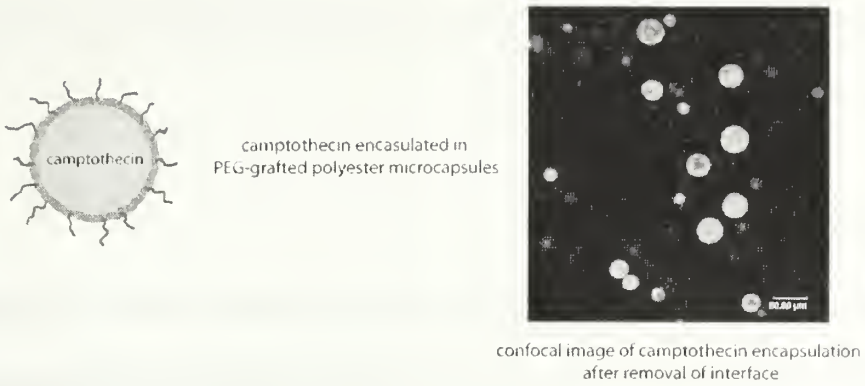


Figure 4.12 Encapsulation of camptothecin within PEG-grafted polyester microparticles including confocal image after removal of the interface

The drug-loaded microparticles were then imaged by confocal microscopy. As the PEG-grafted polyester was not fluorescently labeled for these experiments, the observed fluorescence is solely from encapsulated camptothecin.

These preliminary results demonstrate that click chemistry can be used to prepare cross-linked polyester microparticles that encapsulate hydrophobic drugs and suggest that the cross-link density can be used to control the structural integrity of the microcapsules following removal of the interface. Click chemistry is especially useful for aliphatic polyesters, as acid/base chemistries would not be compatible with the polyester backbone. This system provides a great deal of synthetic flexibility as the cross-link density, number and length of PEG grafts present on the polyester, number of residual acetylene groups available for further functionalization, and length and chemical nature of the diazide cross-linker can all be controlled synthetically and should have dramatic effects on the size, porosity, stability, and degradation of the microcapsules. The choice of oil phase, method of shaking, concentration of polymer, and method for removing the interface will also be important in determining the final

properties. The foundational work described here will provide a starting point for future studies in the Emrick group.

4.3 Future Outlook

In addition to the preparation of cross-linked polyester-microcapsules for drug-delivery the synthetic strategies outlined in this work should afford the opportunity to prepare more complex delivery systems, as depicted in Figure 4.13. For example, by covalently binding one drug to the polyester backbone that comprises a microcapsule its release would be highly dependent upon degradation of the polyester. Meanwhile the release of a second encapsulated unbound-drug would occur by diffusion through the capsule walls thus providing the means for a multi-drug or multi-stage delivery system (Figure 4.13a).

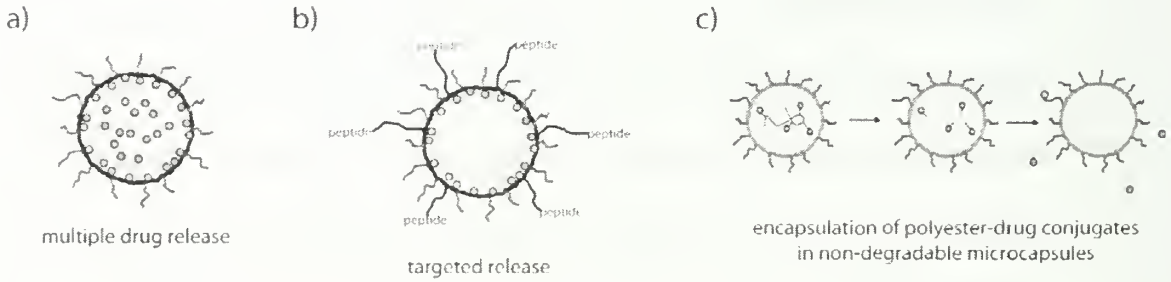


Figure 4.13 Potential applications of PEG-grafted polyester microparticles a) multiple drug release, b) oligopeptide targeted release, and c) encapsulation of hydrophobic polyester-drug conjugates

The incorporation of oligopeptide sequences or other receptor ligands into the microcapsule surface could provide a targeted delivery system with enhanced delivery to specific tissues (Figure 4.13b). Finaly, encapsulation of hydrophobic polyester-drug conjugates in non-degradable microcapsules could afford a long term or multi-stage

delivery system in which release is based on degradation of the polyester-conjugates to a point where they are small enough to escape the capsule by diffusion (Figure 4.13c).

4.4 References

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CHAPTER 5

EXPERIMENTAL SECTION

5.1 General Experimental

- Materials

NCTC clone L929 mouse fibroblasts and Eagle's modified minimum essential medium were purchased from American Type Culture Collection (ATCC). Allyl bromide (99%), 6-bromohexanoic acid (98%), 3-bromopyridine (99%), bromotris(triphenylphosphine)copper(I) (98%), ε-caprolactone (99+%), *N,N'*-dicyclohexyl carbodiimide (DCC) (99%), *N,N*-diisopropylamine (99.5+%), *N,N'*-diisopropylcarbodiimide (DIC) (99%), *N,N*-diisopropylethylamine (≥99%), (4-(dimethylamino)pyridine (DMAP) (99%), Grubbs' second generation ruthenium benzylidene catalyst, hexamethylphosphoramide (HMPA) (99%), 1-hydroxybenzotriazole (HOBt) (<5% water), methanesulfonyl chloride (99.5+%), *N*-methylmorpholine-*N*-oxide (NMO) (50 wt. % solution in water), osmium tetroxide (OsO_4) (4 wt. % solution in water), phenol (99+%), piperidine (99+%), propargyl bromide (80 wt% toluene solution), pyridine (99+%), rhodamine B (90%), sodium azide (99.5%), sodium dodecyl sulfate (99+%), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) (95%), triethylamine (99.5+%), tri(ethylene glycol) monomethyl ether (95%), 2,2,2-trifluoroethanol (TFE) (99.5%), and triisopropylsilane (TIPS) (99%) were obtained from Aldrich. *n*-Butyllithium (2.2M in hexane), *N,N*-diisopropylethylamine (DIPEA) (99%), and tin(II) trifluoromethanesulfonate ($Sn(OTf)_2$) (97%) were purchased from Alfa Aesar; Fmoc-Gly-OH, Fmoc-Ser(But)-OH, Fmoc-Asp(OBut)-OH, Fmoc-

Arg(Pbf)-OH, *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), and Fmoc-Ser(But)-loaded Wang resin (100-200 mesh, loading density of 0.6 mmol/g) were purchased from Advanced ChemTech. Deuterated solvents $CDCl_3$, d_6-DMSO, and D_2O were purchased from Cambridge Isotopes. Silica gel 60 (40-63 μm, 230-400 mesh) was obtained from EM Science. Copper (II) sulfate pentahydrate (ACS reagent grade) and trifluoroacetic acid (TFA) (reagent grade) were purchased from Fisher Scientific. Poly(ethylene glycol) 750 monomethyl ether and poly(ethylene glycol) 1100 monomethyl ether were purchased from Fluka. Sodium ascorbate (crystalline) was purchased from Source Naturals. Dialysis tubing (Spectra/Por® Membrane MWCO 6-8000), horse serum (sterile), phosphate buffered saline (1x w/o Ca or Mg), BD-Falcon® tissue culture flasks (75 cm^2), and BD-Falcon® 6-well plates were obtained from VWR.

Allyl bromide, ε-caprolactone, *N,N*-diisopropylamine, ethanol, HMPA, triethylamine, and δ-valerolactone were distilled over CaH_2 shortly before use. CH_2Cl_2 was washed according to standard procedures[1] and distilled over CaH_2. THF was distilled over sodium/benzophenone. Poly(ethylene glycol) 750 and 1100 monomethyl ethers were purified by column chromatography on silica gel 70 : 20 : 10 CH_2Cl_2 : acetone : methanol to remove diol. All other materials were used without further purification.

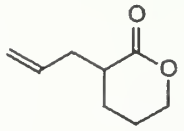
- Instrumentation

NMR spectra were recorded on $CDCl_3$, d_6-DMSO, or D_2O solutions using a Bruker DPX300, Bruker Avance400, or Bruker Avance600 spectrometer (ω_{13C} =

0.25*ω_{111}). Molecular weights and polydispersity indices were measured by gel permeation chromatography in THF or DMF relative to polystyrene standards (Scientific Polymer Products M_p= 503, 700, 1306, 2300, 4760, 12,400, 196,700 and 556,000 g/mol) on systems equipped with three-column sets (Polymer Laboratories 300 x 7.5 mm, 5 μm, 10^{-5}, 10^{-4}, and 10^{-3} Å pore sizes) and refractive-index detectors (HP 1047A) at room temperature (THF) and 50 °C (DMF) with a flow rate of 1 mL/min. High resolution mass spectral (HRMS) data were obtained on a JEOL JMS700 MStation. UV/vis absorbance measurements were taken on a Perkin Elmer Lambda 25 UV/vis spectrometer. IR absorbance data was obtained on a Perkin Elmer Spectrum One FT-IR spectrometer equipped with a universal ATR sampling accessory. Melting points where measured on a Mettler DSC822 differential scanning calorimeter under $N_{2(g)}$ at a scan rate of 10 °C/min, reporting data for the second heating. Dynamic light scattering experiments were conducted using an ALV/SP-125 spectrometer equipped with an ALV-5000/E multiple-tau digital time correlator. A Coherent Innova laser with a wavelength of 514.5 nm was used as the light source, and the temperature of the sample holder was held constant at 25±0.1°C by a circulating water bath. Polyester samples were dissolved in water collected from a Mill-Q UF system at Ω 18.2, to give a concentration of 5.0 mg/ml, and set aside for 24 h. to allow for complete dissolution. Several dilutions of these stock solutions were then made to give final concentrations of 5.0, 2.5, 1.25, and 0.25 mg/ml, and the samples were filtered prior to analysis to remove dust particles (Millipore, hydrophilic PVDF membrane, 0.22μm).

5.2 Synthetic procedures

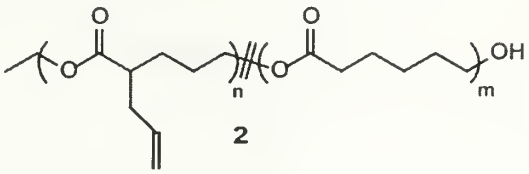
Synthesis of α-allyl-δ-valerolactone (1)



1

A solution of N,N-diisopropylamine (13.8 mL, 99.0 mmol) in THF (700 mL) was stirred under nitrogen and cooled in a dry ice/acetone bath. *n*-Butyllithium (35.4 mL, 99.0 mmol) was added by syringe, and the resulting solution was stirred for 10 min. Then, a solution of δ-valerolactone (8.4 mL, 90 mmol) in THF (200 mL) was added drop-wise over 1.5 h. This mixture was stirred for an additional 30 min. and allyl bromide (9.30 mL, 108 mmol) in HMPA (18.9 mL, 108 mmol) was added drop-wise. The reaction mixture was then warmed to approximately -40 °C (dry ice/acetonitrile bath) and stirred for 2 h. The mixture was quenched with sat. $NH_4Cl_{(aq)}$ (15 mL), and allowed to warm to room temperature. Volatiles were removed by rotary evaporation, and the resulting product dissolved in ether, washed with brine, further dissolved in hexane, and washed with brine again. Column chromatography (gradient 0-15% EtOAc in hexane) followed by Kugelrohr distillation gave lactone **1** as a viscous liquid (8.95 g, 71%): ^{1}H NMR δ 5.75 (m, 1H), 5.06 (m, 2H), 4.26 (m, 2H), 2.55 (m, 2H), 2.26 (m, 1H), 2.04 (m, 1H), 1.85 (m, 2H), 1.54 (m, 1H) ppm; ^{13}C NMR δ 174.0, 135.2, 117.6, 68.6, 39.4, 35.6, 24.21, 22.0 ppm in accord with literature values.[2]

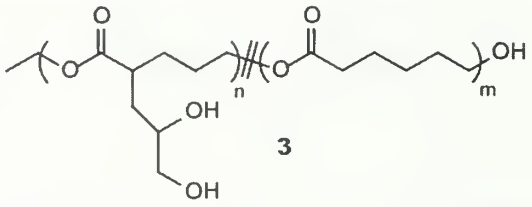
Copolymerization of lactone 1 with ε-CL (Polymer 2_a)



2

A small reaction vessel was thoroughly flamed under a stream of $N_{2(g)}$. Ethanol (1.7M in THF) (80 μL, 1.3 x 10^{-1} mmol) and $Sn(OTf)_2$ (3.7 x 10^{-2} M in THF) (70 μL, 2.7 x 10^{-3} mmol) were added sequentially to the reaction vessel and stirred for 15 min. Lactone **1** (0.84 g, 6.0 mmol) and ε-CL (0.23g, 2.0 mmol) were then added to achieve a feed ratio of (25 : 75 allyl-VL : ε-CL) and degree of polymerization (DP = 60). The reaction mixture was stirred for approximately 24 h. Once conversion had proceeded to >90%, the polymer was dissolved in acetone and precipitated into excess hexanes. Residual functionalized monomer was removed using a plug of silica gel. Monomer was flushed through using 75:25 hexane:ethyl acetate followed by acetone to elute the product. Removal of solvent under reduced pressure afforded polymer $\mathbf{2}_a$ as a clear viscous liquid (1.00 g, 93%): M_n 5700 g/mol, PDI 1.15, ^{1}H NMR δ 5.71 (m, CH_2=C*H*), 5.08 (m, CH_2=CH), 4.06 (m, -CH_2-O lactone **1** and ε-CL), 2.33 (br m, -C=OC*H*-, C=OCHCH_2CH=CH_2, and C=OCH_2-), 1.62 (m, CH_2 lactone **1**, ε-CL), 1.35 (m, CH_2 ε-CL) ppm; ^{13}C NMR δ 175.2 (br, C=O lactone **1**), 173.8 (br, C=O ε-CL), 135.2, 117.6, 64.0, 44.8, 36.5, 34.4, 28.8, 28.6, 26.5, 26.0, 24.9 ppm.

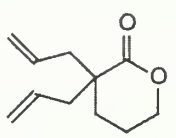
Dihydroxylation of pendent allyl functionality (Polymer 3_a)



3

Polymer 2_a (0.50 g, 7.5 x 10^{-1} mmol allyl functionality), containing 21% incorporation lactone **1**, was stirred in acetone (4 mL). To this mixture was added a 50 wt. % solution of NMO (0.18 g, 7.5 x 10^{-1} mmol) in H_2O. A 1 wt % solution of OsO_4 (0.19 g, 7.5 x 10^{-3} mmol) in H_2O was then added, and the mixture was stirred for at room temperature 24 h. The resulting viscous liquid was washed with water and brine, precipitated into hexanes, and then dried over $MgSO_4$. Volatiles were removed by rotary evaporation to yield **polymer 3a** as a viscous liquid (0.51 g, 87%): M_n 6000 g/mol, PDI 1.13; ^{1}H NMR δ 4.02 (m, 2H), 3.65 (m, 2H), 3.40 (m, 1H), 2.69 (br, 1H), 2.51 (br, 1H), 2.29 (m, 2H), 1.59 (m, 4H), 1.35 (m, 2H); FTIR ν 3457 (O-H), 1725 (C=O) cm^{-1}

Synthesis of α,α-diallyl-δ-valerolactone (4)

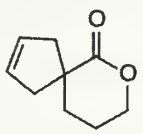


4

N,N-Diisopropylamine (12.3 mL, 88.0 mmol) was dissolved in dry THF (600 mL) and cooled to –78 °C under nitrogen. n-Butyllithium (31.4 mL, 88.0 mmol) was added via syringe and stirred for 15 min. A solution of lactone **1** (7.4 mL, 80 mmol) in dry THF (200 mL) was then added drop-wise over 1 h. The resulting solution was

stirred for 30 min., then a solution of allyl bromide (8.3 mL, 96 mmol) in hexamethylphosphoramide (16.7 mL, 96.0 mmol) was added drop-wise over 20 min. The reaction mixture was then allowed to warm to approximately –30 °C, stirred for 2 h., then quenched with excess aqueous ammonium chloride. Volatiles were removed by rotary evaporation and the resulting product dissolved in ether, washed with brine, diluted with hexane, washed again with brine, dried over $MgSO_4$ and concentrated. Column chromatography (10% EtOAc in hexane) on silica gel followed by Kugelrohr distillation afforded lactone **4** as a clear viscous liquid (13.0 g, 90%); ^{1}H NMR ($CDCl_3$) δ 5.75 (m, 2H, C*H*=CH_2), 5.11 (m, 4H, CH=C*H*$_2$), 4.29 (t, 2H, C*H*$_2$O), 2.55 (d-d, 2H, CC*H*$_2$CH), 2.21 (d-d, 2H, CC*H*$_2$CH), 1.83 (br, 4H, CC*H*$_2$CH$_2$ + CCH$_2$C*H*$_2$) ppm; ^{13}C NMR ($CDCl_3$) δ 175.0 (*C*=O), 133.2 (*C*H=CH_2), 119.2 (CH=*C*H$_2$), 70.3 (*C*H$_2$O), 45.8 (*C*C=O), 43.8 (C*C*H$_2$CH), 28.5 (C*C*H$_2$CH$_2$), 21.0 (CH$_2$*C*H$_2$CH$_2$) ppm.

Synthesis of α-cyclopentene-δ-valerolactone (5)

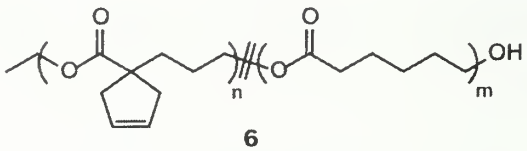


5

Lactone **4** (8.50 g, 47.2 mmol) was dissolved in dry CH_2Cl_2 (200 mL) and [(H_2IMes)(3-Br-py)$_2$($Cl)_2$Ru=CHPh], prepared according to the literature,[2] (40 mg, 4.5 x 10^{-2} mmol) was added as a solution in dry dichloromethane (5 mL). The reaction mixture was refluxed under nitrogen for 4 hours at which point the solvent was removed by rotary evaporation. Lactone **5** was isolated as a clear viscous liquid by Kugelrohr distillation (6.41 g, 90%); HRMS-EI (m/z): M^+ calcd for $C_9H_{12}O_2$, 152. 084;

found, 152. 083; ^{1}H NMR ($CDCl_3$) δ 5.61 (s, 2H, C*H*=C*H*), 4.38 (t, 2H, CH_2O), 3.03 (d, 2H, CCH_2CH), 2.36 (d, 2H, CCH_2CH), 1.89 (br-m, 4H, CCH_2CH$_2$ + CCH$_2$CH_2) ppm; ^{13}C NMR ($CDCl_3$) δ 177.2 (*C*=O), 127.6 (*C*H$_2$=CH$_2$), 69.8 (*C*H$_2$O), 47.7 (*C*C=O), 46.3 (C*C*H$_2$CH), 33.9 (C*C*H$_2$CH$_2$), 20.8 (CH$_2$*C*H$_2$CH$_2$) ppm.

Synthesis of cyclopentene functionalized copolymers (polymer 6a)

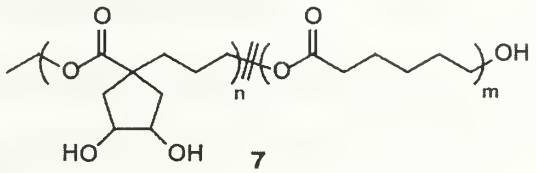


6

All glassware was thoroughly flamed under a stream of $N_{2(g)}$. Stock solutions of ethanol and Sn(OTf)$_2$ were prepared in dry THF (1.7 M and 3.7 x 10^{-2} M respectively). Ethanol (310 μL, 5.3 x 10^{-1} mmol) and Sn(OTf)$_2$ (580 μL, 2.1 x 10^{-2} mmol) were added to the reaction tube and stirred for 15 min. Lactone **5** (970 mg, 6.4 mmol) and ε-CL (2.92 g, 25.6 mmol) were then introduced via syringe. The polymerization was then stirred at room temperature for 48 h. The resulting copolymer was purified by silica gel plug, eluting first with 20% ethyl acetate in hexane to remove residual monomer and then flushing the copolymer through with acetone to afford polymer **6a** as a white semi-crystalline solid (3.77 g, 97%, 14% incorporation of lactone **5**; M_n 7,500, PDI 1.15; ^{1}H NMR ($CDCl_3$) δ 5.59 (s, 2H, C*H*=C*H* lactone **5**), 4.05, (m, CH_2O lactone **5** and ε-CL), 2.83 (d, CH_2CH=CH lactone **5**), 2.27 (m, CH_2C=O $_{CL}$, CH_2CH=CH lactone **5**), 1.64 (br m, CCH_2CH_2 lactone **5**, C*H*2CH2COO ε-CL), 1.39 (br m, CH_2CH$_2$CH$_2$O ε-CL) ppm; ^{13}C NMR ($CDCl_3$) δ 177.3 (*C*=O lactone **5**), 173.6 (*C*=O ε-CL), 128.5 (*C*H$_2$=*C*H$_2$ lactone **5**), 64.4 (*C*H$_2$O lactone **5** and ε-CL), 52.1

(C=O*C*(CH_2)$_3$ lactone **5**), 42.4 (C*C*H_2CH lactone **5**), 35.6 (C=O*C*H_2 ε-CL), 34.2 (C=OC*C*H_2 lactone **5**), 31.0 (*C*H_2CH$_2$O ε-CL), 28.4 (C=OCH$_2$CH$_2$*C*H_2 ε-CL), 25.6 (C=OCH$_2$*C*H_2CH$_2$ ε-CL), 24.6 (CCH$_2$*C*H_2 lactone **5**) ppm.

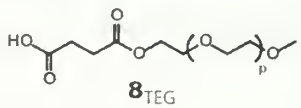
Synthesis of pendant diol copolymers (7)



7

Copolymer **6a** (1.02 g, 0.94 mmol cyclopentene) was dissolved in DMF (5 mL). A 50% weight solution of N-methylmorpholine-N-oxide in water (230 μL, 1.12 mmol) and a 4% weight solution of OsO_4 in water (180 μL, 2.8 x 10^{-2} mmol) were added and stirred vigorously for 8 h. The reaction mixture was then dissolved in CH_2Cl_2 (100 mL), dried over $MgSO_4$, stirred over activated carbon, and filtered through a plug of silica gel. The solvent was then removed to afford copolymer **7a** as a slightly tan colored semi-crystalline solid (980 mg, 93%); M_n 8500 g/mol, PDI 1.15; ^{1}H NMR ($CDCl_3$) δ 4.05 (m, C*H*$_2$O lactone **5** and ε-CL), 3.78 (m, C*H*OH lactone **5**), 2.29 (m, COC*H*$_2$ ε-CL), 1.63 (br m, CC*H*$_2$C*H*$_2$ lactone **5**, C*H*$_2$CHOH lactone **5**, C*H*$_2$CH$_2$C*H*$_2$CH$_2$O ε-CL), 1.40 (br m, C*H*$_2$CH$_2$CH$_2$O ε-CL) ppm; ^{13}C NMR ($CDCl_3$) δ 177.6 (*C*=O lactone **5**), 173.7 (*C*=O ε-CL), 73.6 (*C*HOH lactone **5**), 65.6 (*C*H$_2$O), 64.4 (*C*H$_2$O), 53.5 (C=O*C*(CH_2)$_3$), 46.4 (*C*H$_2$), 35.7 (*C*H$_2$C=O CL), 34.2 (*C*H$_2$), 31.1 (*C*H$_2$), 28.3 (*C*H$_2$), 25.6 (*C*H$_2$), 24.7 (*C*H$_2$) ppm.

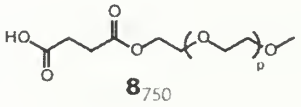
Synthesis of (Tri(ethylene glycol) monomethyl ether)succinic acid ester (8_{TEG})



8_{TEG}

Following the general procedure, the reaction of tri(ethylene glycol) monomethyl ether (5.00 g, 30.5 mmol), succinic anhydride (7.63 g, 76.3 mmol), and DMAP (380 mg, 3.11 mmol) afforded 8_{TEG} as a clear viscous liquid (6.41 g, 80%); ^{1}H NMR ($CDCl_3$) δ 9.49 (br, 1H, COOH), 4.25 (t, 2H, $COOCH_2$), 3.65, (br-m, 8H, OCH_2CH_2), 3.58 (t, 2H, $COOCH_2CH_2$), 3.38 (s, 3H, CH_3), 2.65 (s, 4H, $C{=}OCH_2CH_2$) ppm; ^{13}C NMR ($CDCl_3$) δ 174.2 (*C*OOH), 172.4 (*C*$OOCH_2$), 72.2 (*C*H_2OCH_3), 71.0 ($CH_3OCH_2CH_2OCH_2$), 70.8 ($CH_3OCH_2CH_2$), 70.7 ($CH_3OCH_2CH_2OCH_2CH_2$), 69.3 ($COOCH_2CH_2$), 64.2 ($COOCH_2$), 59.2 (*C*H_3), 29.6 (*C*H_2COOH), 29.3 (*C*H_2CH_2COOH) ppm.

Synthesis of (PEG-750 monomethyl ether)succinic acid ester (8_{750})

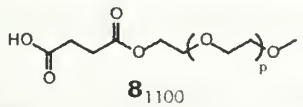


8_{750}

Following the general procedure, the reaction of PEG-750 monomethyl ether (10.0 g, 13.3 mmol), succinic anhydride (3.33 g, 33.3 mmol), and DMAP (165 mg, 1.35 mmol) afforded 8_{750} as a white, waxy solid (10.0 g, 88%); M_n 940, PDI 1.06; ^{1}H NMR ($CDCl_3$) δ 4.24 (t, 2H, $COOCH_2$), 3.64, (br-m, 60H, OCH_2CH_2), 3.56 (t, 2H, $COOCH_2CH_2$), 3.36 (s, 3H, CH_3), 2.66 (br-s, 4H, $C{=}OCH_2CH_2$) ppm; ^{13}C NMR ($CDCl_3$) δ 174.7 (*C*OOH), 172.0 (*C*$OOCH_2$) 72.1(*C*H_2OCH_3), 70.3 (OCH_2CH_2 +

OCH_2CH_2), 68.8 ($COOCH_2CH_2$), 63.6 ($COOCH_2$), 58.8 (CH_3), 28.9 (CH_2COOH), 28.6 (CH_2CH_2COOH) ppm.

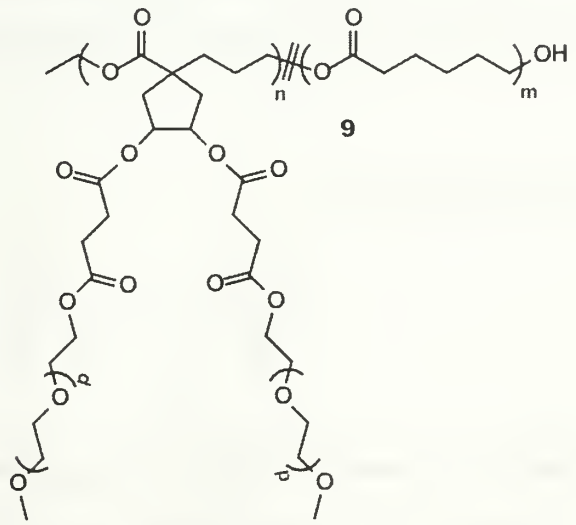
Synthesis of (PEG-1100 monomethyl ether)succinic acid ester (8_{1100})



8_{1100}

Following the general procedure, the reaction of PEG-1100 monomethyl ether (10.0 g, 9.09 mmol), succinic anhydride (2.27 g, 22.7 mmol), and DMAP afforded **8_{1100}** as a white, crystalline solid (8.84 g, 81%); M_n 1,400, PDI 1.05; ^1H NMR ($CDCl_3$) δ 4.24 (t, 2H, $COOCH_2$), 3.63, (br-m, 94H, OCH_2CH_2), 3.36 (s, 3H, CH_3), 2.67 (br-s, 4H, $C=OCH_2CH_2$) ppm; ^{13}C NMR ($CDCl_3$) δ 174.1 ($COOH$), 172.2 ($COOCH_2$), 71.9 (CH_2OCH_3), 70.6 ($OCH_2CH_2 + OCH_2CH_2$), 69.0 ($COOCH_2CH_2$), 63.8 ($COOCH_2$), 59.0 (CH_3), 29.0 (CH_2COOH), 28.9 (CH_2CH_2COOH) ppm.

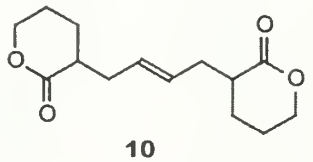
PEG grafting of hydroxyl functionalized copolymers (Polymer 9_{1100})



9

Polymer 7a (500 mg, 0.76 mmol hydroxyl), **PEG-acid 8_{1100}** (1.37 g, 1.14 mmol), and DMAP (7 mg, 8 x 10^{-2} mmol) were dissolved in CH_2Cl_2 (3.5 mL). In a separate flask dicyclohexylcarbodiimide (170 mg, 8.0 x 10^{-1} mmol) was dissolved in pyridine (250 μL, 3.0 mmol) and CH_2Cl_2 (1.5 mL); then added to the reaction vessel. The resulting solution was heated to reflux for 18 h. The reaction mixture was then filtered and the solvent removed by rotary evaporation. Dialysis in 95% ethanol (MWCO 6-8000 tubing, 36 h) yielded **polymer 9_{1100}** as a waxy solid (1.10 g, 78%); M_n 16.5 x 10^3 g/mol, PDI 1.14; ^{1}H NMR ($CDCl_3$) δ 4.24 (m, OCH_2CH_2OCO), 4.05 (m, CH_2OCO), 3.65 (br m, OCH_2CH_2O), 3.37 (s, OCH_3), 2.62 (m, $COCH_2CH_2CO$) 2.30 (m, $COCH_2CH_2CH_2$) 1.64 (m, CCH_2CH_2 lactone **5**, $CH_2CH_2CH_2CH_2O$ ε-CL), 1.40 (br m, $CH_2CH_2CH_2O$ ε-CL) ppm; ^{13}C NMR ($CDCl_3$) δ 177.6 (*C*=O lactone **5**), 173.6 (*C*=O ε-CL), 172.7 (*C*OOCH), 172.3 (*C*$OOCH_2CH_2O$) 71.9 (*C*H_2OCH_3), 70.5 (*C*HOC=O), 69.1 (*C*H_2CH_2O PEG) , 64.2 (*C*H_2O polyester), 63.9 (*C*H_2 PEG), 60.7 (*C*H_2 PEG), 59.0 (*C*H_3), 49.0 (C=O*C*$(CH_2)_3$), 39.8 (*C*H_2 polyester), 34.1 (C=O*C*H_2 CL), 33.9(*C*H_2 polyester), 29.2 (CHC=O*C*H_2CH_2) , 29.1 (CHC=OCH_2*C*H_2), 28.4 (*C*H_2 polyester), 25.7(*C*H_2 polyester), 25.0 (*C*H_2 polyester), 24.6 (*C*H_2 polyester) ppm.

Synthesis of bis-lactone (10)

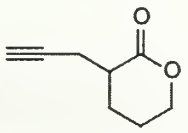


10

Grubbs' "third generation" ruthenium benzylidene catalyst (31 mg, 3.5 x 10^{-2} mmol) was added to a solution of allyl-functionalized lactone **1** (1.0 g, 7.1 mmol) in

CH_2Cl_2 (5 mL) and stirred at room temp. for 6 hours. Solvent was then removed and the crude product purified by column chromatography on silica gel (20% ethylacetate:hexanes) to give bis-lactone **10** as a white crystalline solid (790 mg 72% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 5.51 (m, 2H, (C*H*=C*H*), 4.31 (m, 4H, COOCH_2), 2.53 (m, 2H, C*H*COO), 2.30 (m, 2H, CH_2CH=CH), 2.03 (m, 2H, CH_2CH=CH), 1.90 (m, 4H, $CH_2CH_2CH_2$), 1.60 (m, 4H, C$H_2CH_2CH_2$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 174.3 (*C*=O), 130.2 (*C*H=*C*H), 69.0 (COO*C*H_2), 40.1 (*C*HCOO), 34.7 (*C*H_2CH=CH), 24.6 (*C*$H_2CH_2CH_2$), 22.4 ($CH_2CH_2CH_2$) ppm.

Synthesis of α-propargyl-δ-valerolactone (11)

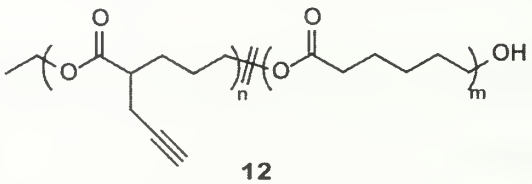


11

n-Butyllithium (42.5 mL, 93.5 mmol) was added by syringe to a solution of N,N-diisopropylamine (13.1 mL, 93.5 mmol) in 625 mL THF at -78 °C and stirred for 15 min. A solution of δ-valerolactone (8.51 g, 85.0 mmol) in 225 mL THF was added drop-wise over 1.5 h and then stirred for an additional 30 min. Propargyl bromide (11.4 mL, 102 mmol) and HMPA (17.7 mL, 102 mmol) were added drop-wise over 20 min. The reaction mixture was then warmed to approximately -30 °C, and the temperature was maintained while stirring for 2 h. Excess aqueous ammonium chloride was added, and the reaction mixture was allowed to warm to room temperature. Volatiles were removed by rotary evaporation. The resulting product was dissolved in ether, washed

with a saturated NaCl aqueous solution, diluted with hexanes, washed again with the NaCl solution, dried over $MgSO_4$, and concentrated. Column chromatography (gradient 0-30% EtOAc in hexanes) on silica gel followed by Kugelrohr distillation afforded lactone **11** as a colorless, viscous liquid (8.68 g, 74% yield). HRMS-EI (m/z): $[M+H]^+$ calculated for $C_8H_{11}O_2$ 139.076, found 139.078. ^1H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 4.28 (m, 2H, C*H*$_2$O), 2.62 (m, 2H, COC*H*C*H*$_2$), 2.46 (m, 1H, COCHC*H*$_2$), 2.22 (sxt, *J* = 6.4 Hz, 1H, CHC*H*$_2$CH2), 1.97 (t-d, *J* = 2.7 Hz *J* = 1.0 Hz, 1H, C≡C*H*), 1.89 (q, *J* = 6.3 Hz, 2H, CH$_2$C*H*$_2$CH$_2$), 1.68 (m, 1H, COCHC*H*$_2$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 172.8 (*C*=O), 81.1 (*C*≡CH), 70.4 (*C*H$_2$O), 68.8 (*C*H≡C), 38.9 (*C*C=O), 24.1 (C*C*H$_2$CH$_2$), 22.0 (CCH$_2$*C*H$_2$), 20.7 (*C*H$_2$C≡CH) ppm. IR(ATR): C≡C-H 3280, C=O 1724 cm^{-1}.

Synthesis of acetylene-functionalized polyesters (polymer 12c)

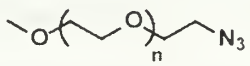


12

Lactone **11** and ε-CL were distilled over CaH_2. Glass reaction vessels were flame-dried three times under a stream of $N_{2(g)}$. EtOH (110 μL, 1.7 M solution in THF) and $Sn(OTf)_2$ (150 μL, 3.7 x 10^{-2} M solution in THF) were introduced to the reaction vessel and allowed to stir for 15 min. Compound **11** (1.10 g, 8.0 mmol) and ε-CL (0.91 g, 8.0 mmol) were then added to the vessel by syringe. The mixture was stirred at room temperature for 48 h and then dissolved in acetone and precipitated into cold hexanes. Residual **11** was removed by passage through a plug of silica gel, eluting with 50:50

EtOAc:hexanes followed by elution of the product with acetone. Solvent was removed by rotary evaporation to give pure polymer **12c** as a clear viscous liquid (1.83 g, 91%). GPC (THF): M_n = 7.5 x 10^3 g/mol, PDI = 1.11. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 4.07 (m, 4H, C*H*$_2$O lactone **11**+CL), 2.58 (m, 1H, C=OC*H* lactone **11**), 2.45 (m, 2H, C*H*$_2$C≡CH lactone **11**), 2.29 (t, *J* = 7.3 Hz, 2H, C=OC*H*$_2$ ε-CL), 2.03 (m, 1H, C≡C*H* lactone **11**), 1.64 (br m, 8H, C*H*$_2$C*H*$_2$CH$_2$O lactone **11** + C*H*$_2$CH$_2$C*H*$_2$CH$_2$O ε-CL), 1.39 (br m, 2H, C*H*$_2$CH$_2$CH$_2$O ε-CL) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 174.1 (*C*=O lactone **11**), 173.6 (*C*=O ε-CL), 81.1 (*C*≡CH lactone **11**), 70.2 (C≡*C*H lactone **11**), 64.6 (*C*H$_2$O lactone **11**), 64.2 (*C*H$_2$OCL), 44.0 (C=O*C*H lactone **11**), 34.2 (C=O*C*H$_2$ ε-CL), 28.4 (*C*H$_2$CH$_2$O ε-CL), 27.6 (CH*C*H$_2$ lactone **11**), 26.2 (CHCH$_2$*C*H$_2$ lactone **11**), 25.6 (C=OCH$_2$CH$_2$*C*H$_2$ ε-CL), 24.6 (C=OCH$_2$*C*H$_2$ ε-CL), 21.3 (*C*H$_2$C≡CH lactone **11**) ppm. IR(ATR): C≡C-H 3283, C=O 1726 cm^{-1}.

Synthesis of PEG-1100 monomethyl ether azide (13)

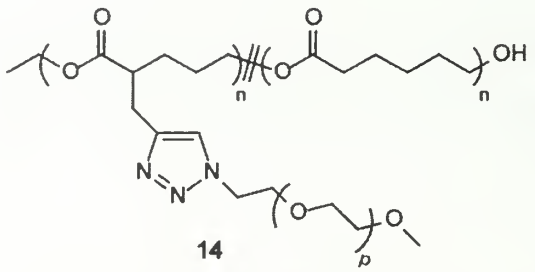


13

PEG-1100 monomethyl ether (10.0 g, 9.09 mmol) and triethylamine (1.5 mL, 10 mmol) were dissolved in THF (50 mL) and cooled to 0 °C. Methanesulfonyl chloride (0.81 mL, 10.5 mmol) was added drop-wise. The reaction mixture was allowed to warm to room temperature and stirred overnight. Solvent was removed by rotary evaporation, and the reaction mixture was dissolved in 95% EtOH (50 mL). Sodium azide (770 mg, 12 mmol) was added, and the reaction mixture was refluxed overnight.

After cooling to room temperature and removing the solvent by rotary evaporation, the crude reaction mixture was dissolved in ether and washed three times with a saturated NaCl aqueous solution. The organic layer was then dried over $MgSO_4$ and concentrated *in vacuo* to give **13** as an off-white crystalline solid (9.10 g, 89% yield). GPC (THF): M_n = 1.2 x 10^3 g/mol, PDI = 1.05. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 3.64 (m, 96 H, CH_2CH_2O) 3.38 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 71.9 (CH_2OCH_3), 70.6 (CH_2CH_2O), 70.0 ($CH_2CH_2OCH_3$), 59.0 (CH_3), 50.6 (CH_2N_3) ppm. IR(ATR): N=N=N 2105 cm^{-1}.

Synthesis of PEG-grafted polyesters of type 14 (polymer 14c)

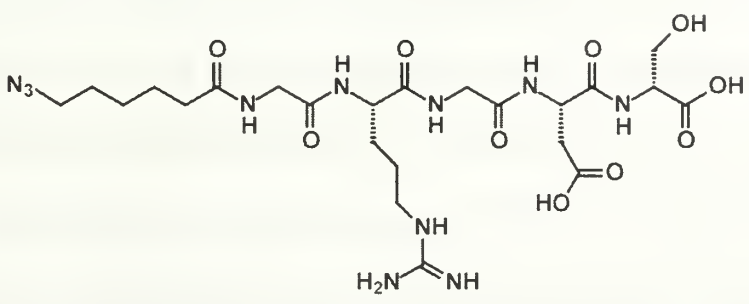


14

PEG-azide **13** (2.20 g, 1.8 mmol) was dissolved in 2 mL of water in a reaction vessel. Polymer **12c** (500 mg, 1.8 mmol acetylene) was dissolved in a minimal amount of acetone and added by syringe to the rapidly stirring reaction mixture. Sodium ascorbate (71 mg, 3.6 x 10^{-1} mmol) and copper (II) sulfate (45 mg, 1.8 x 10^{-1} mmol) were added, and the resulting dispersion was heated to 80 °C overnight. The crude reaction mixture was then diluted with 50 mL of water, and the product was extracted 5 times with dichloromethane. The combined organics were dried over $MgSO_4$ and concentrated by rotary evaporation. Residual **13** was removed by dialysis in PBS. The resulting product was then dissolved in a minimal amount of acetone and precipitated

into cold hexanes to give pure polymer **14c** as an off-white powder (2.1 g, 78%). GPC (THF): M_n = 16.3 x 10^3 g/mol, PDI = 1.13. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 7.49 (s, 1H, R_2C=C*H*), 4.49 (m, 2H, R_2NCH_2), 4.03 (br, 4H, CH_2OC=O lactone **11** + ε-CL), 3.83 (t, *J* = 5.15 Hz, 2H, $R_2NCH_2CH_2$), 3.62 (br m, 96H, CH_2CH_2OPEG), 3.45 (t, *J* = 5.44 Hz, 2H, CH_2OCH_3 PEG), 3.38 (s, 3H, CH_3 *PEG*), 3.00 (br, 2H, R_2CHCH_2 lactone **11**), 2.49 (br, 1H,C=OC*H* lactone **11**), 2.39 (br, 2H, C=OCH_2CL) 1.61 (m, 8H, $CH_2CH_2CH_2O$ lactone **11** + $CH_2CH_2CH_2CH_2O$ ε-CL), 1.38 (br m, 2H, $CH_2CH_2CH_2O$ ε-CL) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 174.4 (*C*=O lactone **11**), 173.6 (*C*=O ε-CL), 144.5 (R_2C=CR), 122.5 (R_2C=*C*R), 71.6 (CH_2OCH_3 PEG), 70.5 (CH_2CH_2O PEG) 69.2 ($R_2NCH_2CH_2O$), 64.6 (CH_2O lactone **11**), 64.2 (CH_2O ε-CL), 58.7 (CH_3 PEG), 53.3 (R_2NCH_2), 49.8 (R_2CHCH_2 lactone **11**), 44.7 (C=O*C*H lactone **11**), 34.2 (C=OCH_2 ε-CL), 28.4 (CH_2CH_2O ε-CL), 27.7 (CHCH_2 lactone **11**), 25.9 ($CHCH_2CH_2$ lactone **11**), 25.6 (C=O$CH_2CH_2CH_2$ ε-CL), 24.6 (C=OCH_2CH_2 ε-CL) ppm.

Synthesis of oligopeptide-azide GRGDS-N_3 (15)



15

The oligopeptide sequence GRGDS was synthesized according to standard Fmoc solid phase peptide synthesis using the batch-wise process and the peptide

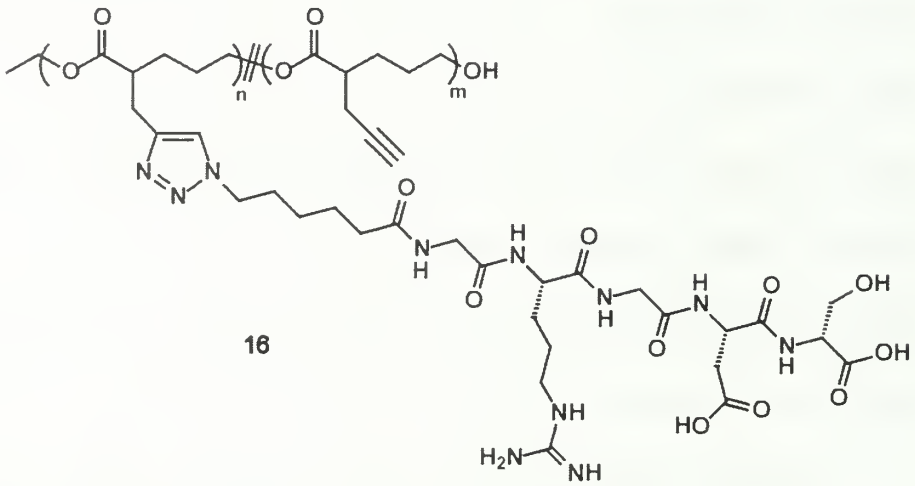
coupling agent HBTU.[3] Fmoc-Ser(But)-loaded Wang resin (3.12 g with loading density of 0.6 mmol/g) was weighed into an oven-dried glass-fritted reaction tube and swollen with 30 mL dry CH_2Cl_2 for 5-10 min. The Fmoc group was cleaved by the addition of a 25/75 solution of piperidine/DMF (30 mL), followed by agitation with $N_{2(g)}$ for 3 min. The resin was filtered, and fresh piperidine/DMF (30 mL) was added. After agitating for 20 min, the resin was filtered and washed with DMF 6 times. A solution of Fmoc-Asp(OBut)-OH (3.85 g, 9.35 mmol), HBTU (3.48 g, 9.17 mmol), and HOBt (1.26 g, 9.35 mmol) in 20 mL of anhydrous DMF was then prepared. After the solution became homogeneous, DIPEA (3.28 mL, 18.70 mmol) was added, and the resulting mixture was added immediately to the resin. The resin was then agitated for 1 h, filtered, and washed with DMF (3 times). A 25/75 solution of piperidine/DMF (30 mL) was added, and the resin agitated for 3 min. After filtration, piperidine/DMF was again added to the resin followed by agitation for 20 min. The resin was then washed with DMF (6 times). The above amino acid addition procedure was repeated for Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, and a second unit of Fmoc-Gly-OH. Following the addition of the second Gly unit, a solution of 6-bromohexanoic acid (1.82 g, 9.35 mmol) and HOBt (1.26 g, 9.35 mmol) was prepared in 15 mL of dry DMF. DIC (1.45 mL, 9.35 mmol) was added drop-wise to the solution and stirred for 20 min. The activated solution was added to the resin and agitated for 1 h. After filtration, the resin was washed with DMF (6 times) followed by CH_2Cl_2 (4 times) to remove any residual DMF. The peptide was then deprotected and cleaved from the resin by agitating with a 88/2/5/5 solution of TFA/TIPS/H_2O/phenol (30 mL) for 3 h. The solution was filtered, and the cleavage procedure was repeated with 30 mL of fresh solution and 30 min agitation. The resin

was then washed with CH_2Cl_2 (3 times), and the filtrated was concentrated by rotary evaporation, precipitated into ether, and stored at 4 °C for several hours before filtration. The solid was isolated by filtration, rinsed with diethyl ether (3 times), and dried under vacuum overnight to afford GRGDS-bromide as a white powder in nearly quantitative yield (based upon the given resin-loading density). HRMS-FAB (m/z): $[M+H]^+$ calculated for $C_{23}H_{39}N_8O_{10}Br$ 667.205, found 667.207. ^1H NMR (d_6-DMSO, 400 MHz): δ (DMSO = 2.50 ppm) 11.90 (br, 2H), 8.30 (br, 1H), 8.20 (d, J = 7.9 Hz, 1H), 8.07 (m, 2H), 7.88 (d, J = 7.7 Hz, 1H), 7.72 (br, 1H), 7.16 (br, 2H), 4.64 (m, 1H), 4.22 (m, 2H), 3.66 (m, 8 H), 3.50 (tr, J = 6.7 Hz, 2H), 3.07 (m, 2H), 2.13 (tr, J = 7.2 Hz, 2H), 1.78 (m, 2H), 1.64 (br, 2H), 1.50 (m, 6H), 1.35 (m, 2H) ppm. ^{13}C NMR (d_6-DMSO, 125 MHz): δ (DMSO = 39.52 ppm) 172.7, 172.0, 171.9, 170.7, 169.4, 168.7, 156.8, 61.4, 55.0, 52.3, 49.4, 42.1, 40.4, 36.4, 35.1, 35.0, 32.1, 29.1, 27.3, 24.9, 24.4 ppm. IR(ATR): O-H and N-H 3286.1, C-H 2937.0, C=O 1644.1, N-H 1531.9 cm^{-1}.

Bromide-terminated GRGDS (1.17 g, 1.75 mmol) was then dissolved in DMSO (3.5 mL, 0.5 M), and NaN_3 (0.13 g, 2.0 mmol) was added to the solution. The reaction was allowed to proceed for 12-18 h at room temperature after which the solution was filtered through Celite. Following rotary evaporation and Kugelrohr distillation to remove DMSO, the crude product was dissolved in a minimal amount of methanol, and the insoluble precipitate was removed by filtration. The remaining solution was precipitated from diethyl ether and filtered to afford GRGDS-azide **15** as a white powder (1.09 g, 99% yield). HRMS-FAB (m/z): $[M+H]^+$ calculated for $C_{23}H_{39}N_{11}O_{10}$ 630.296, found 630.296. ^1H NMR (d_6-DMSO, 400 MHz): δ (DMSO = 2.50 ppm) 8.36 (br, 1H), 8.27 (m, 2H), 8.07 (m, 2H), 7.72 (d, J = 7.4 Hz, 1H), 7.23 (br, 2H), 4.58 (m,

1H), 4.23 (m, 1H), 4.14 (m, 1H), 3.59 (m, 8H), 3.29 (tr, J = 6.8 Hz, 2H), 3.07 (m, 2H), 2.13 (tr, J = 7.3 Hz, 2H), 1.76 (m, 2H), 1.50 (m, 6H), 1.28 (m, 2H) ppm. ^{13}C NMR (d_6-DMSO, 125 MHz): δ (DMSO = 39.52 ppm) 173.4, 172.7, 172.6, 172.4, 170.6, 169.3, 168.8, 157.2, 61.8, 55.3, 52.4, 50.6, 49.7, 42.5, 42.0, 40.5, 36.8, 35.0, 29.4, 28.1, 25.8, 24.7, 24.5 ppm. IR(ATR): O-H and N-H 3261, N=N=N 2097, C=O 1645 cm^{-1}.

Synthesis of oligopeptide-grafted polyesters (polymer 16d)

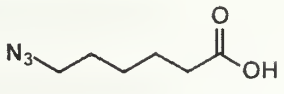


16

Peptide azide **15** (23 mg, 3.6 x 10^{-2} mmol) was dissolved in 2 mL of water and heated to 80 °C. Homopolymer **14d** (100 mg, 7.2 x 10^{-1} mmol acetylene) was dissolved in 1 mL of acetone and added drop-wise to the rapidly stirring solution. Sodium ascorbate (122 mg, 6.1 x 10^{-1} mmol) and copper (II) sulfate pentahydrate (79 mg, 3.2 x 10^{-1} mmol) were then added, and the resulting dispersion stirred at 80 °C until bubbling due to evaporation of acetone had ceased. The reaction vessel was then fitted with a condenser, heated to reflux, and stirred overnight. The reaction mixture was cooled to room temperature, diluted with a saturated NaCl aqueous solution, and extracted 5 times with CH_2Cl_2. The combined organic layers were then dried over $MgSO_4$ and

concentrated by rotary evaporation. The resulting product was dried overnight under vacuum to yield polymer **16d** as a yellow viscous liquid in 50-70% yield. GPC (DMF, after 1 month): Mn = 19.4 x 10^3 g/mol, PDI = 1.07. ^{1}H NMR ($CDCl_3$, 600 MHz): δ ($CHCl_3$ = 7.26 ppm) 6.70-7.80 (m, 6 H), 4.73-4.75 (m, 1 H), 4.30-4.32 (m, 2H), 4.07-4.17 (m, 4 H), 3.64 (tr, *J* = 6.4 Hz, 2 H), 2.73 (m, 2 H), 2.38-2.60 (m, 6H), 2.02 (tr, *J* = 2.5 Hz, 1H), 1.64-1.78 (m, 8H), 1.58 (m, 6H) ppm. IR (ATR): C-H 2944.1, C-H 2864.5, C=O 1721.9 cm^{-1}.

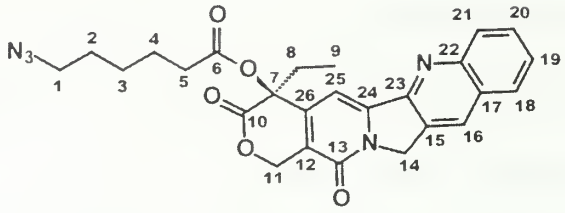
Synthesis of 6-azidohexanoic acid (17)



17

6-Bromohexanoic acid (5.0 g, 25.6 mmol) and sodium azide (8.37 g, 128 mmol) were dissolved in DMSO and stirred at room temp. for 8 hours. The reaction mixture was then dissolved in CH_2Cl_2, washed with water, brine, and $NaHCO_{4(aq)}$, dried over $MgSO_4$ and concentrated by rotary evaporation. Residual DMSO was removed by Kugelrohr distillation at 120 °C. Distillation at 160 °C gave 6-azidohexanoic acid 7 as a colorless liquid (2.98 g, 74%). MS-ESI (m/z): $[M+Na]^+$ calculated for $C_6H_{11}N_3O_2$ 180.1, found 180.1. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 11.43 (br, 1H, COO*H*), 3.28 (t, 2H, C$H_2$$N_3$), 2.38 (t, 2H, C$H_2$COOH), 1.65 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 180.1 (*C*=O), 51.2 (CH_2N_3), 33.9 (CH_2COOH), 28.5 ($CH_2CH_2N_3$), 26.2 ($CH_2CH_2CH_2N_3$), 24.2 (CH_2CH_2COOH) ppm. IR(ATR): N=N=N 2090 cm^{-1}.

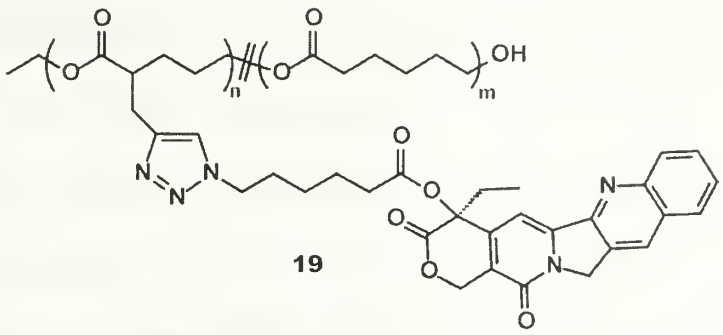
Synthesis of camptothecin-azide (18)



18

Camptothecin (100 mg, 2.7 x 10^{-1} mmol) 6-azidohexanoic acid **17** (85 mg, 5.4 x 10^{-1} mmol) and DMAP (4 mg, 3 x 10^{-2} mmol) were dissolved in CH_2Cl_2 (5 mL). DCC (110 mg, 5.4 x 10^{-1} mmol) was added and the solution stirred at room temp. for 8 hours. The reaction mixture was then precipitated into diethyl ether, filtered, and recrystallized from MeOH:CH_2Cl_2 95:5 to give camptothecin-azide **18** as a yellow, crystalline solid (114 mg, 87%). HRMS-EI (m/z): $[M]^+$ calculated for $C_{26}H_{25}N_5O_5$ 487.186, found 487.186. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 8.40 (s, 1H, C-16*H*), 8.23 (d, *J* = 8.4 Hz, 1 H, C-18*H*), 7.95 (d, *J* = 8.4 Hz, 1H, C-21*H*), 7.84 (t, *J* = 6.8 Hz, 1H, C-19*H*), 7.67 (t, *J* = 7.6 Hz, 1H, C-20*H*), 7.21 (s, 1H, C-25*H*), 5.70 (d, *J* = 17.2 Hz, 1H, C-11H_2), 5.43 (d, *J* = 17.2 Hz, 1H, C-11H_2), 5.29 (s, 2H, C-14*H*), 3.22 (t, *J* = 6.8 Hz, 2H, C-1H_2) 2.51 (m, 2H, C-5H_2), 2.28 (m, 1H, C-8H_2) 2.16 (m, 1H, C-8H_2), 1.67 (m, 2H, C-2H_2), 1.62 (m, 2H, C-4H_2), 1.43 (m, 2H, C-3H_2) 0.98 (t, *J* = 7.2 Hz, 2H, C-9H_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 172.5 (*C*=O lactone C-10), 167.7 (*C*=O ester C-6), 157.5, 152.5,149.0, 146.4, 146.0, 131.4, 130.9, 129.7, 128.6, 128.4, 128.3, 128.2, 120.4, 96.0, 75.9, 67.2, 51.3 (CH_2N_3 C-1), 50.1, 33.7, 32.0, 28.6, 26.2, 24.3, 7.7 (CH_3 C-9) ppm. IR(ATR):N=N=N 2096 cm^{-1}.

Synthesis of polyester-camptothecin conjugate (polymer 19a)

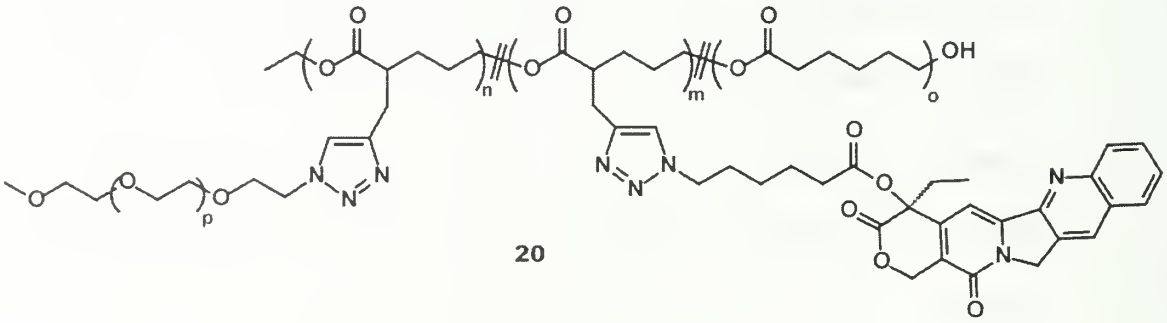


19

Polymer $\mathbf{12_e}$ (10 mole percent acetylene, M_n 7.6 x 10^3 g/mol, PDI 1.18, 120 mg, 1.0 x 10^{-1} mmol acetylene) was dissolved in CH_2Cl_2 (2 mL) in a small reaction vessel. Camptothecin-azide **18** (49 mg, 1.0 x 10^{-1} mmol), *N,N*-diisopropylethylamine (7.0 μl, 4.0 x 10^{-2} mmol), and bromotris(triphenylphosphine)copper(I) (19 mg, 2.0 x 10^{-2} mmol) were then added sequentially and the reaction mixture was stirred at room temp. for 48 h. The crude reaction mixture was precipitated into ether, purified by dialysis in CH_2Cl_2, and then precipitated into hexanes to afford polymer $\mathbf{19_a}$ as an off-white semi-crystalline solid (140 mg, 83%). GPC (THF): M_n = 11.9 x 10^3 g/mol, PDI = 1.18. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 8.39 (s, 1H, camptothecin **18** C-16*H*), 8.23 (d, *J* = 8.3 Hz, 1 H, camptothecin **18** C-18*H*), 7.93 (d, *J* = 8.3 Hz, 1H, camptothecin **18** C-21*H*), 7.86 (t, *J* = 6.9 Hz, 1H, camptothecin **18** C-19*H*), 7.64 (m, 2H, R_2C=C*H* + camptothecin **18** C-20*H*), 7.19 (s, 1H, camptothecin **18** C-25*H*), 5.65 (d, *J* = 15.2 Hz, 1H, camptothecin **18** C-11H_2), 5.39 (d, *J* = 15.3 Hz, 1H, camptothecin **18** C-11H_2), 5.29 (s, 2H, camptothecin **18** C-14*H*), 4.25 (br, 2H, C$H_2$$NR_2$ camptothecin **18**), 4.04 (br, 4H, CH_2OC=O lactone **11** + ε-CL), 3.22 (t, *J* = 6.6 Hz, 2H, camptothecin **18** C-1H_2), 3.01 (br, 2H, R_2CHCH_2 lactone **11**), 2.51 (m, 2H, camptothecin **18** C-5H_2), 2.35 (br, 4H,C=OC*H* lactone **11** + C=OCH_2 CL + camptothecin **18** C-8H_2), 2.15 (m, 1H,

camptothecin **18** C-8H_2), 1.65 (br m, 12H, CH_2CH_2CH_2O lactone **11** + CH_2CH_2CH_2CH_2O ε-CL + camptothecin **18** C-2H_2 + camptothecin **18** C-4H_2), 1.36 (br m, 4H, CH_2CH_2CH_2O ε-CL + camptothecin **18** C-3H_2), 0.99 (t, J = 7.1 Hz, 2H, camptothecin **18** C-9H_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 174.2 (*C*=O lactone **11**), 173.4 (*C*=O $_{CL}$), 172.5 (*C*=O camptothecin **18** C-10), 167.6 (*C*=O camptothecin **18** C-6), 157.5, 152.5, 149.0, 146.4, 146.0, 144.5, 131.4, 130.9, 129.7, 128.6, 128.3, 128.3, 128.2, 122.5, 120.4, 96.0, 75.9, 67.2, 64.6, 64.1, 53.3, 50.1, 49.9, 44.7, 34.1, 33.7, 32.1, 28.6, 28.4, 27.6, 26.2, 25.9, 25.4, 24.5, 24.3, 7.7 (*C*H_3 camptothecin **18** C-9) ppm.

Synthesis of PEGylated polyester-camptothecin conjugate (polymer 20a)



20

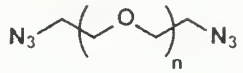
Polymer **19$_b$** was prepared according to the procedure for polymer **19$_a$** by coupling of polymer **12$_f$** (52 mole percent acetylene, M_n 9.2 x 10^3 g/mol, PDI 1.17, 500 mg, 2.1 mmol acetylene) with camptothecin-azide **18** (150 mg, 3.1 x 10^{-1} mmol) in the presence of *N,N*-diisopropylethylamine (23 μl, 1.2 x 10^{-1} mmol) and bromotris(triphenylphosphine)copper(I) (57 mg, 6.2 x 10^{-2} mmol) to target 15 mole percent camptothecin grafting. Purification by precipitation into ether followed by dialysis in CH_2Cl_2 afforded polymer **19$_b$** as a slightly yellow viscous liquid (580 mg,

89%). GPC (THF): M_n = 14.3 x 10^3 g/mol, PDI = 1.19, 12 mole percent camptothecin grafting, 38 mole percent residual acetylene as determined from ^{1}H NMR spectroscopy by the relative integrations of signals at 4.25 (C*H*$_2$NR$_2$ camptothecin **18**), 4.04 (C*H*$_2$OC=O polyester backbone), 2.03 (-C≡C-H).

Polymer **19$_b$** (580 mg, 1.6 mmol acetylene) was dissolved in a minimal amount of acetone and added by syringe to a rapidly stirred solution of PEG-azide **13** (1.90 g, 1.6 mmol) in water (2 ml). Sodium ascorbate (130 mg, 6.4 x 10^{-1} mmol) and copper (II) sulfate (80 mg, 3.2 x 10^{-1} mmol) were added, and the resulting dispersion was heated to 80 °C overnight. The crude reaction mixture was then diluted with 50 mL of water, and the product was extracted 5 times with CH_2Cl_2. The combined organics were dried over $MgSO_4$ and concentrated by rotary evaporation. Residual **13** was removed by dialysis in PBS. The resulting product was then dissolved in a minimal amount of acetone and precipitated into cold hexanes to give polymer **20** as an off-white powder (1.80 g, 74%). GPC (THF): M_n = 23.5 x 10^3 g/mol, PDI = 1.20. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 8.36 (s, 1H, camptothecin **18** C-16*H*), 8.20 (d, *J* = 8.3 Hz, 1 H, camptothecin **18** C-18*H*), 7.95 (d, *J* = 8.3 Hz, 1H, camptothecin **18** C-21*H*), 7.85 (t, *J* = 6.7 Hz, 1H, camptothecin **18** C-19*H*), 7.66 (m, 2H, R_2C=C*H* + camptothecin **18** C-20*H*), 7.51 (s, 1H, R_2C=C*H*), 7.21 (s, 1H, camptothecin **18** C-25*H*), 5.64 (d, *J* = 14.9 Hz, 1H, camptothecin **18** C-11*H*$_2$), 5.40 (d, *J* = 14.9 Hz, 1H, camptothecin **18** C-11*H*$_2$), 5.26 (s, 2H, camptothecin **18** C-14*H*), 4.46 (m, 2H, R_2NCH_2), 4.23 (br, 2H, C*H*$_2$NR$_2$ camptothecin **18**), 4.02 (br, 4H, C*H*$_2$OC=O lactone **11** + ε-CL), 3.80 (t, *J* = 5.15 Hz, 2H, $R_2NCH_2CH_2$), 3.61 (br m, 96H, C*H*$_2$C*H*$_2$O PEG), 3.43 (t, *J* = 5.44 Hz, 2H, C*H*$_2$OCH$_3$ PEG), 3.37 (s, 3H, C*H*$_3$ PEG), 3.20 (t, *J* = 6.6 Hz, 2H, camptothecin **18** C-1*H*$_2$), 3.00

(br, 2H, R_2CHCH_2 lactone **11**), 2.53 (m, 2H, camptothecin **18** C-5H_2), 2.34 (br, 4H,C=OC*H* lactone **11** + C=OCH_2 ε-CL + camptothecin **18** C-8H_2), 2.16 (m, 1H, camptothecin **18** C-8H_2), 1.66 (br m, 12H, $CH_2CH_2CH_2O$ lactone **11** + $CH_2CH_2CH_2CH_2O$ ε-CL + camptothecin **18** C-2H_2 + camptothecin **18** C-4H_2), 1.35 (br m, 4H, $CH_2CH_2CH_2O$ ε-CL + camptothecin **18** C-3H_2), 0.97 (t, J = 7.1 Hz, 2H, camptothecin **18** C-9H_3) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm) 174.1 (*C*=O lactone **11**), 173.3 (*C*=O ε-CL), 172.5 (*C*=O camptothecin **18** C-10), 167.7 (*C*=O camptothecin **18** C-6), 157.5, 152.3, 149.1, 146.4, 146.0, 144.5, 131.3, 130.9, 129.7, 128.6, 128.2, 128.3, 128.2, 122.5, 120.4, 96.0, 75.9, 71.7 (CH_2OCH_3 PEG), 70.4 (CH_2CH_2O PEG), 69.2, 67.2, 64.7, 64.1, 58.6 (CH_3 PEG), 53.3, 50.1, 49.8, 44.7, 34.0, 33.7, 32.0, 28.6, 28.4, 27.5, 26.2, 25.9, 25.4, 24.5, 24.3, 7.8 (CH_3 camptothecin **18** C-9) ppm.

Synthesis of PEG-diazide cross-linker(21)

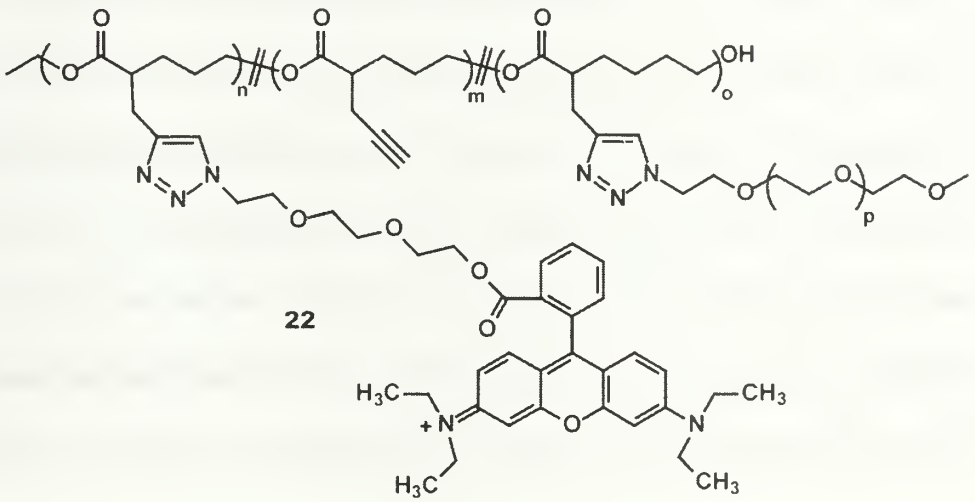


21

PEG-200 (5.0 g, 25 mmol) and triethylamine (9.38 mL, 62.5 mmol) were dissolved in THF (200 mL) and cooled to 0 °C. Methanesulfonyl chloride (4.83 mL, 62.5 mmol) was added drop-wise. The reaction mixture was allowed to warm to room temperature and stirred overnight. Solvent was removed by rotary evaporation, and the reaction mixture was dissolved in 95% EtOH (200 mL). Sodium azide (8.17 mg, 125 mmol) was added, and the reaction mixture was refluxed overnight. After cooling to room temperature and removing the solvent by rotary evaporation, the crude reaction

mixture was dissolved in ether and washed three times with $NaCl_{(aq)}$. The organic layer was then dried over $MgSO_4$ and concentrated *in vacuo* to give **21** as a slightly yellow, viscous liquid (5.50 g, 87% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ ($CHCl_3$ = 7.26 ppm) 3.64 (m, 16 H, CH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ ($CHCl_3$ = 77.0 ppm), 70.6 (CH_2CH_2O), 70.0 (CH_2CH_2O), 50.6 (CH_2N_3) ppm. IR(ATR): N=N=N 2110 cm^{-1}.

Synthesis of rhodamine B-labeled PEGylated polyesters (22)



Mono-azide functionalized triethylene glycol was prepared according to the literature.[4] The mono-azide (74 mg, 0.42 mmol), rhodamine B (100 mg, 0.21 mmol), and DMAP (5 mg, 4 x 10^{-2} mmol) were dissolved in 2 mL CH_2Cl_2. DCC (87 mg, 0.42 mmol) was then added and stirred at room temp. for 8 hours. The reaction mixture was then diluted with excess CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated by rotary evaporation. Efforts to obtain a pure compound were hindered by impurities contained in the starting material. However, after dialysis was performed

in water PBS for several days, most of the fluorescent impurities were removed and the sample was deemed suitable for imaging by fluorescent confocal microscopy.

5.3 Cytotoxicity Testing

Cell culture

NCTC clone L929 mouse fibroblasts were purchased from American Type Cell Culture (ATCC) and cultured according to standard aseptic techniques.[5] The adherent fibroblasts were grown on BD-Falcon® polystyrene tissue culture flasks (75 cm^2) in modified Eagle's minimal essential medium (EMEM) also purchased from ATCC. This medium contains Earle's balanced salt solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and was supplemented with 10% horse serum. Cells were incubated at 37 °C in a 5% CO_2 atmosphere, and the medium was replaced with fresh medium every 3 days. Once the adherent cells reached >90% confluence they were removed from the surface with trypsin, counted using a hemacytometer, and reseeded at a density of 1×10^5 cells/cm^2.

MEM evaluation

NCTC clone L929 mouse fibroblasts were seeded at a density of 1×10^4 cells/cm^2, in BD-Falcon® 6-well polystyrene plates, to reach near confluence in 48 h. After 24 h incubation, the medium was removed and replaced with fresh medium. For cytotoxicity evaluation, polymer samples dissolved in 300 μL phosphate buffered saline (PBS) were introduced to the cells along with the media to give a final concentration of 5 mg/mL polymer. As positive and negative controls respectively, 300 μL PBS and 300

μL PBS containing sodium dodecyl sulfate (SDS) to give a final concentration of 5 mg/mL, were added separately to cells along with media (all experiments were performed in triplicate). Once the material was added, the fibroblasts were incubated for an additional 24 h and then observed microscopically for changes in confluence and morphology. The cells incubated with polymer samples were compared qualitatively to the positive control in respect to cell density and the number of rounded versus spread cells.

Hemolysis

Human whole blood was extracted via vacuum syringe at the UMASS Health Center and stored in EDTA stabilized vials at 4 °C until use (for a maximum of 1 week). The red blood cells (RBCs) were separated from plasma by 4 repeated cycles of centrifugation, removal of the supernatant, and resuspension in sterile PBS. The RBCs were diluted once more in PBS to give a 10% w/v suspension. The material to be tested was dissolved in 100 μL PBS, introduced to 900 μL of the RBC suspension, and incubated at 37 °C for 30 min. Samples were then centrifuged, and the absorbance of the supernatant was measured at 413 nm. RBC suspension to which only 100 μL PBS was added provided a baseline value that was subtracted from all other values. RBC suspension incubated with 100 μL deionized water was used to determine 100% lysis. All other values are reported as a relative percentage, and each value is the average for three samples.

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